# **BIOMATERIALS** AND SUSTAINABLE FUTURE

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Editor: Assoc. Prof. Dr. Elif URAL

Marmara Üniversity

eozenural@marmara.edu.tr

https://orcid.org/0000-0001-8447-7161

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mail: destek@artikelakademi.com www.artikelakademi.com

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#### WRITERS

Ahmet ACARER Emek ASLAN Emircan GÜNGÖR Eray ARSLAN Meral ÖZOMAY Semra ÜNAL Shahid ADEEL Zafer ÖZOMAY



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#### From The Editor,

This book has been prepared to contribute to the efforts of producing nature-friendly solutions in the context of global climate change and sustainability. With contributions from experts in various fields, we aim to provide an in-depth understanding of topics that hold great importance for humanity. This distinctive work, supported by significant efforts and international research by all our authors, encompasses studies on climate change, biodiversity, and natural dyes. I believe this book, consisting of five chapters, will serve as a guiding light for a sustainable future.

In the first chapter, we discuss the negative consequences of climate changes from the formation of the Earth to the present day, as well as the efforts made to mitigate these challenges. The development of scenarios, which are among the most critical components of climate modeling, is also elaborated upon. Furthermore, the chapter emphasizes how a better understanding of the impacts of climate change on both nature and humanity facilitates the development of effective intervention strategies. The second chapter explores eco-friendly approaches to bioproducts and plant protection in sustainable agriculture. Various bioproducts are meticulously examined, with a focus on their applications and effectiveness. The third chapter delves into the cultivation of algal biomass, a foundation for numerous value-added productions, and examines cultivation techniques that ensure high biomass efficiency. It provides valuable insights into the optimization and/or correlation and combination of different elements within two distinct techniques. The fourth chapter presents scientific data demonstrating the use of collagen in textile dyeing. Type I collagen, derived from animal bones, is examined. Additionally, the chapter highlights how collagen significantly enhances flame retardancy. The fifth chapter discusses bacterial cellulose, a multifunctional Nano biomaterial composed of hydrogen-bonded and linear glucan molecules, resembling plant cellulose. It is noted that, compared to cotton fabric, bacterial cellulose exhibits superior results in color yield and other fastness tests.

These studies on combating climate change, biotechnology, and sustainable production techniques are expected to raise awareness not only in the academic realm but also in agriculture, industry, and other sectors of society.

I wish you an enlightening read.

Assoc. Prof. Dr. Elif URAL

### CHAPTER 1

#### **EMERGENCE OF GLOBAL CLİMATE CHANGE**

#### **Dr. Ahmet ACARER**

Isparta University of Applied Sciences, Faculty of Forestry, Department of Wildlife Ecology and Management, Isparta/Turkey aacarer32@gmail.com, https://orcid.org/0000-0003-0864-7880

#### **1. INTRODUCTION**

In the period from the beginning of world history to today, the geographical features of the world have changed several times and the disruptions in the natural balance have caused great changes in the climate (Öztürk, 2002). While these changes varied with the spread and retreat of glaciers, the negative effects of human activities towards the end of the 19th century also caused an increase in climate change (Adamo et al., 2021). To eliminate the negative effects of this climate change or to overcome it with minimal damage, researchers from different disciplines have concentrated on studies on topics such as greenhouse gases, global warming or global climate change (Ramanathan and Feng, 2009; Cao et al., 2016). In fact, the issue of "climate change" that may occur due to CO2 emissions was first put forward in Switzerland in 1896 (Stehr, 2013). Although it was put forward in 1896, climate change was ignored for a long time (Hulme, 2009). At the World Climate Conference held in 1979, the importance of the climate change process was underlined and was described as the first important breakthrough (Oldfield, 2018). At this conference, it was emphasized that the

accumulation of CO2 in the atmosphere will increase with the increase in fossil fuels and forest use (Graham et al., 1990).

After the World Climate Conference, meetings by scientists became continuous and the issue of climate change became the focus of attention for researchers. Therefore, the information presented in the World Climate Conference symposium has been reinforced in events such as conferences, meetings and symposiums held by researchers from different scientific disciplines. However, due to disagreements among this information, the issue of climate change has become a difficult issue to accept on a global scale. Therefore, regional and global reports have been prepared among different non-governmental organizations, researchers, public institutions and even states (Clark, 1995). The most important point in these reports put forward by different institutions and organizations is the increasing effects of climate change on a global scale (Adger et al., 2005). It is predicted that the effects of this climate change will directly or indirectly affect living things and even reach a level where they become extinct, and the necessity of taking certain precautions has been emphasized (NRC, 2013).

The Intergovernmental Panel on Climate Change (IPCC) was established in 1988 with the aim of identifying the negative impacts of climate change and implementing necessary measures, through the collaboration of countries affiliated with the United Nations (Change, 1995). This panel plays a crucial role under the auspices of the World Meteorological Organization and the United Nations Environment Programme in assessing the potential consequences of climate change caused by human activities (Tamiotti, 2009). The IPCC provides forecasts about the future impacts of climate change through its reports, which are published in specific years, and offers recommendations for necessary mitigation measures. The most recent report, known as the Sixth Assessment Report, was made publicly available in 2022 following preliminary work that began in 2016. This report contains critical information for combating climate change (Eckstein et al., 2021; Dwivedi et al., 2022).

The reports presented by the IPCC are based on three main factors: scientific foundation, compliance studies, and mitigation measures. These approaches rely on global climate models (GCMs) to implement necessary measures and make predictions for future years. Global climate models (GCMs) play a critical role in conducting statistical assessments related to climate (Raäisaänen, 2007; Hartmann, 2016). By analysing various parameters in the oceans, land surface, and atmosphere, these models determine how the climate responds to current <sup>10</sup>

greenhouse gas emissions and are among the most advanced systems available. However, the analysis of these systems and the derivation of results require a complex process. Nevertheless, the potential to make future predictions and implement protective measures makes accepting this complexity worthwhile (Eyring et al., 2016; Pottier et al., 2017).

One of the most critical components of climate modelling efforts is the development of scenarios. The Special Report on Emission Scenarios (SRES), prepared within the framework of the IPCC's Fourth Assessment Report published in 2007, has served as a vital reference point for numerous studies and has been extensively utilized in modelling activities. The SRES comprises four main scenarios (A1, A2, B1, B2) along with various subcategories (Van Vuuren et al., 2007; Aerts et al., 2013). The development of SRES scenarios began in 1997 and was completed after approximately three years of rigorous work. While initial findings were presented in 2001, comprehensive results were only made available in the Fourth Assessment Report published in 2007 (Parry, 2007). These scenarios provide an essential framework for identifying possibilities related to climate change and for informing strategic decision-making (Hallegatte, 2009).

The development of SRES scenarios employs a methodology known as the "sequential approach". This approach divides the scenario creation process into a series of interrelated phases. In the first phase, socio-economic scenarios are formulated; subsequently, emission scenarios that reflect the socio-economic developments are generated (Holman et al., 2005). Finally, based on the identified emission levels, the radiative forcings caused by these emissions are calculated, and the resulting data are incorporated into climate models (Özkan and Mert, 2010). The four primary emission scenarios presented within the SRES framework are listed below.

#### **1.1. SRES Climate Scenarios**

#### 1.1.1. A1 Scenario

The A1 scenario is founded on the premise of a homogeneous worldview characterized by rapid economic growth, significant technological advancements by the century's end, intensified socio-cultural interactions, and a peak in global population. According to this scenario, CO2 emissions are expected to rise from 380 ppm in 2000 to 800 ppm by 2080, which would correlate with an average temperature increase of approximately 3 °C (IPCC, 2007; Nunez et al., 2009). The A1 scenario class is subdivided into three distinct categories: A1F1, which emphasizes a system heavily reliant on fossil fuels; A1T1, which represents a scenario devoid of fossil fuel usage; and A1B, which envisions a balanced approach to energy resource utilization (IPCC, 2007; Schweizer and Kriegler, 2012; IPCC, 2013).

#### 1.1.2. A2 Scenario

In contrast, the A2 scenario depicts a heterogeneous world where local identities are maintained, per capita income is lower, and technological progress is relatively slow (Kaivo-oja et al., 2004). This scenario anticipates continued population growth, projecting that CO2 levels will rise from 380 ppm in 2000 to 700 ppm by 2080, accompanied by a temperature increase of around 2.8 °C (Bernstein et al., 2008; Bosetti and Frankel, 2009; Özkan and Mert, 2010).

#### 1.1.3. B1 Scenario

The B1 scenario represents the most optimistic outlook within this group, forecasting a future marked by economic development while highlighting the importance of ecological measures by the end of the century (Riahi et al., 2007; MacCracken, 2008). It envisions a reduction in pollutant sources and anticipates that CO2 concentrations will reach 580 ppm by 2080, with a corresponding temperature increase of approximately 1.8 °C (IPCC, 2007; Özkan and Mert, 2010).

#### 1.1.4. B2 Scenario

The B2 scenario, as one of the SRES types, presents a heterogeneous worldview like that of the A2 scenario (De La Cueva et al., 2012). However, it predicts a lower population growth compared to A2, alongside moderate economic development. Additionally, the B2 scenario forecasts a slower technological advancement than the B1 and A1 scenarios. Specifically, it is anticipated that CO2 concentrations, which were 380 ppm in 2000, will rise to 550 ppm by 2080, resulting in an approximate temperature increase of 2.1 °C (IPCC, 2007; Ainsworth and McGrath, 2010).

The Fourth Assessment Report provided a comprehensive framework by presenting various scenarios with differing projections regarding climate change (Suppiah et al., 2007). The IPCC regularly updates these scenarios to ensure the accuracy and relevance of climate change information. For instance, in the Fifth Assessment Report, new scenario groups were introduced that differ from the SRES scenarios (Stocker, 2014). In developing these new climate change scenarios, a parallel assessment method was employed, diverging from the sequential approach used in the earlier SRES scenarios (Moss et al., 2010; Van Vuuren et al., 2011). The newly developed concentration scenarios are referred to as Representative Concentration Pathways (RCPs) and, like the SRES scenarios, consist of four main scenarios (RCP 2.6, 4.5, 6.0 and 8.5) based on radiative forcing levels (Baek e al., 2013; IPCC, 2013; Van Vuuren and Carter, 2014; Eyring et al., 2016). Below, potential concentration and temperature projections associated with the RCP scenarios are presented.

#### **1.2. RCP Climate Scenarios**

#### 1.2.1. RCP 2.6

RCP 2.6 stands out as the most optimistic scenario within the RCP framework (Chou et al., 2014). Under this projection, CO2 concentrations are expected to peak at 442 ppm by 2050 (Millar et al., 2017). Following this peak, the implementation of ecological and environmental protection measures is anticipated to lead to a decline in CO2 levels, ultimately reaching 421 ppm by the century's end. Moreover, the average temperature is projected to rise by approximately 1.0 °C (minimum: 0.3 °C; maximum: 1.7 °C) during this timeframe (Demircan et al., 2014; Shrestha and Lohpaisankrit, 2016).

#### 1.2.2. RCP 4.5

In the RCP 4.5 scenario, driven by technological advancements and population growth, CO2 emissions are forecasted to continue increasing throughout the century, reaching 538 ppm by 2100 (Thomson et al., 2011). The expected rise in temperature is approximately 1.8 °C (minimum: 1.1 °C; maximum: 2.6 °C) (GDM, 2013).



#### 1.2.3. RCP 6.0

Similarly, the RCP6.0 scenario predicts that CO2 concentrations will rise in tandem with increasing population and industrialization, reaching 670 ppm by 2100. This scenario anticipates a temperature increase of around 2.2 °C (minimum: 1.4 °C; maximum: 3.1 °C) (Demircan et al., 2014; Eyring et al., 2016; Shrestha and Lohpaisankrit, 2016).

#### 1.2.3. RCP 8.5

According to the RCP8.5 scenario, ongoing population growth combined with intensified industrial activities is expected to lead to a significant increase in CO2 levels, reaching 936 ppm by 2100. The projected temperature rise under this scenario is approximately 3.7 °C (minimum: 2.6 °C; maximum: 4.8 °C) (Ayala et al., 2016; Eyring et al., 2016;)

The latest climate change scenarios released by the IPCC are encapsulated in the 6th Assessment Report. This set of scenarios, referred to as "21st Century Scenarios", was made accessible to researchers in the first quarter of 2022 (O'Neill et al., 2016). The newly established framework, known as "Shared Socioeconomic Pathways (SSP)," integrates socioeconomic modelling with the objectives of the RCP scenarios, offering five distinct pathways for addressing climate change (Carbonbrief, 2024). These foundational approaches are further categorized into alternative subgroups based on radiative forcing values as applied in various global climate models. Global climate models are defined as intricate mathematical representations of the interactions among key components of the climate system, including the atmosphere, land surface, oceans, and sea ice. These models utilize projections of these components to simulate climate dynamics and anticipate future scenarios. The SSP scenarios delineate potential future pathways as follows: SSP 1 envisions a world centered on sustainable development and equity; SSP 2 reflects a middle-ground trajectory largely aligned with historical trends; SSP 3 portrays a fragmented world marked by a resurgence of nationalism; SSP 4 characterizes a landscape of increasing inequality; and SSP 5 illustrates a scenario of rapid and unrestricted economic growth. Detailed information regarding these scenarios is provided below (Eyring et al., 2016; O'Neill et al., 2016; Kebede et al., 2018; Özdemir et al., 2020; WorldClim, 2023; Carbonbrief, 2024; IIASA, 2024).



#### 1.3 SSP Scenario

### **1.3.1.** SSP 1: Low Level Challenges for Mitigation and Adaptation (*Sustainability – Following the Green Path*)

In this scenario, the world is undergoing a significant transformation towards a more sustainable future by focusing on environmental factors. This process is progressing in parallel with the increasing awareness of environmental issues among societies, leading to more widespread approaches to the conservation of natural resources. On a global scale, governance mechanisms are evolving into more inclusive structures that encourage public participation, thereby enhancing the importance placed on human-centered values. As a result, the balance between economic growth and individual well-being is being prioritized, emphasizing that human dignity extends beyond mere economic gains to include a commitment to social justice. This transformative process also contributes to the reduction of inequalities, both within and among countries. Steps taken towards social services and equal opportunities lay the groundwork for a more equitable world order. Furthermore, consumption patterns among individuals and communities are shifting; energy and resource usage is becoming increasingly efficient. In this context, sustainable energy solutions and environmentally friendly practices are becoming central to economic activities, fostering significant advancements towards the goal of leaving a healthier planet for future generations (Eyring et al., 2016; O'Neill et al., 2016; Özdemir et al., 2020; WorldClim, 2023; Carbonbrief, 2024; IIASA, 2024).

## **1.3.2.** SSP 2: Moderate Challenges for Mitigation and Adaptation (*Middle of the Road*)

This scenario outlines a roadmap for the world, following historical patterns of economic, technological, and social dynamics. Development and income growth are proceeding with marked inequality among countries; while some nations are making significant progress, others are struggling to meet their targets. National and international organizations are undertaking various initiatives to achieve sustainable development goals, but this process is not advancing at the desired pace. Although there have been some improvements in energy and resource use, the threats to ecosystems are increasingly pronounced. Global population

growth is expected to slow down in the second half of the century. However, the rising income inequality casts a shadow over this optimistic outlook. The ability of societies to respond to social and environmental changes continues to pose significant challenges. In this context, there is a pressing need for more effective and comprehensive strategies to ensure sustainable development. International cooperation is critical for achieving equitable resource distribution and social justice. This approach would not only support economic growth but also enhance the protection of ecosystems, thereby increasing the chances of building a more sustainable future. The success of this process hinges on the collective efforts and responsibilities of all stakeholders involved (Eyring et al., 2016; O'Neill et al., 2016; Özdemir et al., 2020; WorldClim, 2023; Carbonbrief, 2024; IIASA, 2024).

## **1.3.3.** SSP 3: High Challenges for Mitigation and Adaptation (Regional Competition – A Rocky Road)

This scenario depicts a situation characterized by a resurgence of nationalism and competitive ideologies. Security concerns and escalating regional conflicts compel nations to increasingly focus on their internal affairs and external relations. In this context, policymakers prioritize addressing national and regional security issues, shaping their strategic planning accordingly. Countries are intensifying their efforts to achieve energy and food security as part of their economic development goals. Achieving these objectives necessitates a focus on self-sufficiency in agriculture and energy production. However, the declining investment in education and technological development poses a significant threat to long-term development targets. Economic growth is stagnating, raw material consumption is on the rise, and social inequalities are persisting and even deepening. Population growth remains low in industrialized countries, while it is notably high in developing nations. This disparity generates varying impacts on economic and social dynamics, leading to significant changes in labour markets across many countries. The rising population exacerbates resource consumption and increases environmental pressures. Additionally, this demographic shift intensifies the demand for social services, although meeting these demands often proves challenging. There is a discernible decline in international sensitivity to ecological living conditions. Discussions surrounding environmental sustainability frequently lag security and economic development priorities, contributing to the escalation 16

of environmental degradation. In certain regions, clear signs of ecological deterioration are emerging, indicating that essential ecosystem services, such as agriculture, water resources, and biodiversity, are under threat. In conclusion, this scenario illustrates a world grappling with serious challenges on both national and international fronts. Fundamental issues, such as security, energy, and food, occupy priority positions among state objectives, while the risk of deviating from long-term sustainable development goals increases. Therefore, adopting a multifaceted and holistic approach is crucial for addressing these global challenges. Without effective international collaboration and solidarity, progress in resolving these issues is likely to remain limited. Hence, fostering effective communication and cooperation among governments, civil society organizations, and international institutions becomes an essential requirement for a sustainable future (Eyring et al., 2016; O'Neill et al., 2016; Özdemir et al., 2020; WorldClim, 2023; Carbonbrief, 2024; IIASA, 2024).

# 1.3.4. SSP 4: Low Difficulties in Mitigation, High Difficulties in Adaptation *(Inequality – A Divided Path)*

In this scenario, the inequalities in investments in human capital, combined with the growing disparities in economic opportunities and political power, lead to an increasing stratification both within and among countries. Over time, the gap between societies contributing to the knowledge and capital-intensive sectors of the global economy and those characterized by low income, low education, and labour-intensive employment continues to widen. This situation contributes to a decline in social cohesion and a rise in societal unrest. Investments in technology show a tendency to increase. However, these investments are directed not only toward carbon-intensive fuels such as coal and oil but also toward low-carbon energy sources. This contradictory situation presents a complex picture for environmental sustainability. Environmental policies typically focus on local issues in middle- and high-income regions, which may result in the neglect of environmental challenges in low-income areas. Consequently, environmental inequalities are exacerbated, and issues related to the unfair use of natural resources come to the forefront. This poses significant threats to both social justice and environmental sustainability. In summary, these processes lead to increased societal unrest and weaken the bonds among individuals, ultimately creating a dynamic that threatens the integrity of communities. For a sustainable future, it is crucial to take steps not only in economic and environmental policies

but also in ensuring social cohesion and justice (Eyring et al., 2016; O'Neill et al., 2016; Özdemir et al., 2020; Carbonbrief, 2024).

## **1.3.5.** SSP 5: High Level Challenges in Mitigation, Low Level Challenges in Adaptation (*Fossil Fuel Development – Taking the Highway*)

In this scenario, there is a strong emphasis on the development of human capital within the framework of sustainable development paradigms. In this context, competitive marketing strategies, rapidly evolving technological advancements, and innovative approaches contribute significantly to the participatory and inclusive growth of societies. As global markets become increasingly integrated, substantial investments in health and education play a crucial role in strengthening social capital. At the same time, the rising pressures for economic and social development are intertwined with dynamics that promote the use of fossil fuels and the adoption of energy-intensive lifestyles worldwide. While these processes drive the rapid growth of the global economy, projections indicate that the world population will peak in the mid-21st century and then begin to decline. Local environmental issues, such as air pollution, are being effectively managed through advanced governance strategies, thereby alleviating concerns regarding environmental sustainability. This scenario fosters a robust belief in the effective management of both social and ecological systems, enabling societies to pursue healthier and more balanced ways of life. Furthermore, as human-centered approaches are embraced, the critical importance of aligning economic growth with social justice and environmental protection is underscored. In conclusion, this scenario presents an essential framework for achieving the balance necessary for future generations to thrive in a liveable world. In this context, it is imperative for societies to unite their individual and collective efforts to attain sustainable development goals. By doing so, a future will be constructed where not only economic gains, but also social and environmental well-being are prioritized (Eyring et al., 2016; O'Neill et al., 2016; Özdemir et al., 2020; Carbonbrief, 2024; Bellis et al., 2024.).

The scenarios outlined above provide insights into various alternative futures presented within the framework of the Shared Socioeconomic Pathways (SSP). Among these scenarios, particularly SSP 1 and SSP 5 predict significant investments in education and healthcare, alongside rapid economic growth and the presence of effective governance structures. However, the stark differences



between these two scenarios are noteworthy. SSP 1 emphasizes a transition towards sustainable development goals, proposing a growth model that considers environmental and social balance, while SSP5 assumes a continuation of energy-intensive, fossil fuel-based economic growth, potentially overlooking the environmental consequences of such an approach. Conversely, SSP 3 and SSP 4 highlight the inadequacies of investments in education and healthcare in low-income countries, along with increasing population pressures and deepening socioeconomic inequalities, thus painting a more pessimistic picture regarding future economic and social developments. These scenarios suggest that deteriorating socioeconomic conditions may contribute to societal unrest and the proliferation of conflicts. SSP 2, representing a "middle-of-the-road" scenario, predicts that historical developmental patterns will persist throughout the 21st century. This scenario encompasses both optimistic and pessimistic elements, reflecting the complexity of social and economic dynamics. Consequently, it establishes a significant quest for balance in achieving international cooperation, sustainability efforts, and social cohesion. In conclusion, these scenarios offer a comprehensive framework for understanding the challenges and opportunities faced by countries. Insights drawn from these diverse scenarios are crucial for the development of policies and strategies aimed at achieving sustainable development goals. Thus, the SSP scenarios provide essential foresight into how future social, economic, and environmental dynamics may unfold, assisting decision-makers in crafting more informed and effective strategies (Eyring et al., 2016; O'Neill et al., 2016; Özdemir et al., 2020; Carbonbrief, 2024).

When analysing the scenarios, it becomes evident that the SSP (Shared Socioeconomic Pathways) scenarios share notable similarities with the SRES (Special Report on Emissions Scenarios) scenarios. For instance, the sustainable development-oriented SSP1 closely aligns with the SRES B1 scenario. Both scenarios emphasize the importance of investments aimed at enhancing social welfare and achieving environmental sustainability, while prioritizing improvements in education and health. Similarly, SSP2 demonstrates significant parallels with SRES B2. Both scenarios focus on maintaining historical development patterns and achieving a balance between social and economic factors. However, this focus may also entail risks of exacerbating social inequalities and environmental issues. SSP3, which anticipates a fragmented global structure, shares characteristics with SRES A2. Both scenarios indicate that increasing international competition and security concerns will lead nations



to concentrate more on domestic issues, potentially hindering economic and social development and exacerbating societal unrest. On the other hand, SSP5, which predicts a high dependency on fossil fuels, shares common features with SRES A1F1. These scenarios suggest that the role of carbon-intensive fuels in energy consumption will increase, highlighting the adverse effects on climate change. However, the approach to CO2 emissions within the SSP scenarios distinctly differs from that of the SRES scenarios. SSPs offer a more flexible and dynamic framework, allowing for the potential integration of emission reduction strategies. In conclusion, the SSP scenarios provide a comprehensive analytical framework that encompasses not only the current state but also potential future pathways. This presents significant opportunities for policymakers and researchers to develop more effective strategies regarding climate change mitigation, sustainable development, and social equity. The flexibility and integrative nature of the SSPs, compared to the SRES scenarios, play a crucial role in shaping the future. Thus, it becomes possible to target a more just, sustainable, and environmentally compatible future for societies (Eyring et al., 2016; O'Neill et al., 2016; Özdemir et al., 2020; Carbonbrief, 2024).

#### CONCLUSION

The scenarios developed by the Intergovernmental Panel on Climate Change (IPCC) serve as essential tools for shaping our understanding of the potential future impacts of climate change. These scenarios are utilized to analyse the dynamics of the complex climate system and to predict the consequences of climate change under various potential futures. Numerous studies from different disciplines employ these scenarios to make forward-looking predictions regarding specific target variables and to develop strategic responses. Particularly noteworthy are studies focusing on natural ecosystems, such as ecology, biodiversity, forestry, and agriculture. Research in these areas highlights the dramatic transformations induced by climate change within natural ecosystems and the associated threats to ecosystem health. For instance, global temperature increases, alterations in the water cycle, and extreme weather events are reshaping habitats for various plant and animal species, posing significant risks to biodiversity. Therefore, scenarios related to climate change are critically important for understanding how natural systems may evolve in the future. Furthermore, the data provided by these



scenarios constitute a vital information resource for policymakers and scientists. In this context, the scenarios used in climate change research reveal not only the future status of natural ecosystems but also the wide-ranging consequences affecting human health, food security, and economic stability. Consequently, the significance of these studies extends beyond merely elucidating the effects of climate change on nature; they also provide crucial insights for developing strategies on how human societies can adapt to these changes. In summary, these comprehensive investigations based on different years and scenarios are essential for understanding the dynamics, impacts, and potential outcomes of global climate change. This section is critical in providing the necessary information for better comprehending the effects of climate change on both nature and human life, thereby facilitating the development of effective intervention strategies. The scenarios presented by the IPCC serve as an indispensable reference point in this study to inform the fight against climate change and the development of adaptation strategies.

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### CHAPTER 2

#### BIOPRODUCTS: THE GREENER APPROACH FOR PLANT PROTECTION IN SUSTAINABLE AGRICULTURE

Assist. Prof. Dr. Emek ASLAN

Ondokuz Mayıs University, Faculty of Agriculture Department of Agricultural Biotechnology emek.aslan@omu.edu.tr https://orcid.org/0000-0002-1287-3322

### 1. INTRODUCTION TO PLANT PROTECTION IN AGRICULTURE

Sustainable agricultural practices have successfully emerged from the thoughtful integration of plant protection into various agroecosystems. The innovative use of bioproducts is a promising alternative to conventional agrochemicals in plant protection. Applying these controlled, carefully monitored methods yields an impact on field production levels. It significantly contributes to the commercial development of high-value crops critical to local and global markets (Giraldo et al., 2023). Bioproducts encompass diverse technologies and strategies, including biological control methods, host resistance techniques, and the utilization of naturally occurring bioactive chemicals such as plant extracts, essential oils, pheromones, and practices like intercropping. Furthermore, developing and implementing natural ecosystems as valuable sources of beneficial species are crucial in enhancing agricultural resilience and sustainability

(Ashokkumar et al., 2022; Priya et al., 2023). The urgent need for practical plant protection systems to meet the escalating global food demands resulting from demographic growth in the forthcoming years necessitates a sustained effort to persistently introduce and adopt new sustainable practices across the agricultural landscape (Sodhi et al., 2022; Jain et al., 2022). In the scope of this chapter, various bioproducts will be meticulously reviewed and contextualized within the framework of plant protection across diverse agroecosystems. Each bioproduct will be analyzed in terms of its application and effectiveness. Additionally, an indepth discussion regarding their functionality will be provided and several cost and benefit considerations that merit attention.

#### 1.1. Importance of Plant Protection in Sustainable Agriculture

The growing demand for agricultural products faces limitations related to the increased costs and the problems generated by the current conventional agriculture model. There is a broad consensus that current farming practices are not sustainable due to their inherent negative impacts that result in negative externalities (Panchasara et al., 2021). Traditional agriculture, based on the green revolution technologies adopted since the 1960s, has been able to guarantee an increase in food productivity and, consequently, to minimize the consumption of land and forests in a direct ratio to the rise in population and to the need for feeding this increase in a way that was proportional to productivity has decreased food scarcity for many of the population, this has been somewhat eclipsed by the negative environmental impacts. The green revolution biological products - herbicides, pesticides, and chemical fertilizers - account for 35% of greenhouse gas (GHG) emissions and deep environmental impoverishment responsible for 70% of the water and land used by humanity (Benavides et al., 2020).

Sustainable agriculture tries to minimize these negative externalities. It can be expressed as creating and integrating elements of design, techniques, and technologies into a system that quickly offers minimum adverse environmental impact (Edwards, 2020). The goal is to aim at accomplishment of the constant creation of food products, the correct use of the resources needed for its production (earth, soil, water, and air), guaranteeing the compensation of those who act in this sense, according to the association of public and private stakeholders, and ensuring that other living beings are players in the process. The agribusiness sector must innovate in management and marketing strategies to come closer to the community, acting sustainably and adding ethical responsibility to its business. This approach is considered an antifragile model, capable of adapting and learning quickly, creating opportunities more effectively and efficiently than its sectorial competitors (Muhie, 2022). The risks associated with the sustainability of the agricultural system are too high, given its dimensions, renewal rate of natural resources, and population growth. After all, the consequences are unpredictable, and the system is fragile to external incentives, with a value death spiral to ensure lanes of corporate security profitability. Agriculture must follow, in this sense, its dynamic and flexible capacity of adaptation, together with the evolution of global society (Siebrecht, 2020; Wang et al., 2022).

#### 1.2. Challenges in Conventional Plant Protection Methods

Plant production is continuously damaged by many microbial plant pests, with viruses, bacteria, and fungi being the most prevalent. The high percentage of plants susceptible to pests and the long evolution of the pests, causing an efflux of pesticides with acquired resistance to a broad spectrum of organisms, have been important factors that make us reflect on sustainable agriculture. Differential tolerance of agricultural techniques for similar phenotypic species can determine the rate of competitiveness. Many pests, viruses, bacteria, and fungi injure these food and ornamental plants. It is believed that the number of pests affecting agribusiness worldwide exceeds 8000. (Secretariat et al., 2021; Seppelt et al., 2022)

The great majority of plant protection products in agriculture are chemical products that are harmful to the environment and human health, resulting in residues linked to the excessive use of these products. As a result, many biological, botanical, and microbiological products have been developed in recent years to be used as biopesticides for plant protection (Thakur et al., 2020). There is a growing demand for chemical-free, organic, and food-safety crops. Organic/natural pesticides and biological growth promoters are playing an increasingly important role in the sustainable development of agricultural research (Samada & Tambunan, 2020). The beneficial microorganisms in these products can favor and promote plant growth. We must increase our understanding

of the mechanisms involved in effective soil-efficiency relationships, including beneficial plant-microbe interactions, at the plant-soil interface. This knowledge can be used to build new management tools. Since the plant benefits must be tangible and essential, the commercial success of emerging solutions depends on well-documented efficiency. Integrating these innovative products can improve agricultural sustainability by promoting long-term soil fertility, plant health, and crop production practices that maintain economic profitability (Khursheed et al., 2022; Kumar et al., 2021).

#### 2. BIOPRODUCTS: DEFINITION AND CLASSIFICATION

Bioproducts are substances used in agriculture to manage and protect crops. In the particular case of plant protection, 96 bioproducts, including products obtained by fermentation with genetically modified microorganisms, are products of a biological nature, namely composed of or obtained from microorganisms or of plant, animal, or mineral origin, including non-genetically modified organisms. Biological substances and products are infinitely diverse in structure, action mode, and origin. They have been known and used for centuries, principally in traditional agriculture. (Singh & Yadav, 2020; Ashokkumar et al.2022; Priya et al., 2023)

Native microorganisms, such as bacteria, fungi, oomycetes, viruses, bacteriophages, and nematodes, maintain and restore biological equilibrium with algorithms to protect ecologically balanced agroecosystems. Naturally occurring predators, invertebrate pathogens, and parasitic microorganisms are among the natural factors attacking aggressive or harmful reserve organisms (French et al., 2021). These would include plant and livestock pathogens and all their specific enemies, in particular, herbivorous populations and phytophagous populations; specific fungi, oomycetes, bacteria, viruses, nematodes, insects, or mites; nesting on or within them, attacking the same farm or plant at different times of the year. (Koskey et al.2021; Bhaduri et al., 2022; Coban et al., 2022).

#### 2.1. Biopesticides

The global economic pressure for rapid advancement in the field of agriculture, primarily aimed at feeding the increasingly large world population, <sup>30</sup>



coupled with the significant challenges presented by declining agricultural production environments, has led to the emergence of a diverse array of tools and agents designed to address the constraints present in the production sector effectively. These challenges have become increasingly pressing as the need for innovative solutions becomes more urgent (Thakur et al., 2020; Kumar et al., 2021). Additionally, the potential threat posed by such advancements to existing sustainable production environments has sparked widespread calls for "breathing space" to ensure that these new techniques can coexist harmoniously, or even be fully integrated, within current agricultural systems. The plant protection sector emerges as a vital component in agrarian science, recognized for its crucial role in successfully realizing these ambitious aims. Experts widely accept that a more holistic and comprehensive approach to developing new methods and paradigms for manipulating agricultural outcomes is beneficial and necessary within the plant protection domain. This paper presents a compelling argument illustrating how biopesticides already occupy a key strategic position within the new emphasis on integrated organic agriculture and biotechnology. These biopesticides hold promise in creating a new agricultural paradigm that promotes sustainability while addressing the demands of modern food production (Hernández-Fernández et al., 2021; Pathma et al., 2021).

The term "biopesticide" has only been used since 1988, yet the formulations and various ingredients that are part of this category are far from new; many have been utilized for centuries as specific remedies aimed at targeting pest species without causing harm to other organisms. Their defining characteristics include safety, target specificity that ensures they focus on particular pest species, and the ability to effectively disrupt the interactions between pests and their targets (Kumar et al., 2021). Throughout the 19th and most of the 20th century, biopesticides were primarily neglected, as the agricultural industry favored the adoption of broad-spectrum agrochemicals. These chemicals typically offered curative capabilities rather than preventive measures of farm practices, which often favored a more straightforward management approach relying predominantly on chemical treatments, routine prophylactic applications, and a limited array of modes of activity represented by each ingredient in use. Despite their previous relegation, the reintroduction of biopesticides and their gradual expansion in the commercial market now hinges on two significant considerations. The first of these concerns is the growing controversy regarding the human and environmental safety associated with some of these formerly



trusted agents, which raises important questions about their use. The second consideration is the increasing awareness of the potential these biopesticides hold for enhancing returns on investment, particularly in the rapidly evolving fields of biotech research and development. Since their inception, the concepts driving these critical concerns have intertwined with the foundational ideas of integrated, organic, and sustainable agriculture (Akutse et al., 2020; Agboola et al., 2022; Chakraborty et al., 2023). The scientific principles underlying the use of biopesticides have been revised and reinforced using contemporary tools within informatics, biotechnology, ecology, and the sciences dedicated to crop protection.

#### 2.2. Biostimulants

Biostimulants are products that, when applied to plants or the rhizosphere, act to stimulate natural processes that enhance and benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, and crop quality traits, in addition to advancing germination without acting against the crop itself or the surrounding environment. Among diverse nutrient management tools, biostimulants with nutrient sources can increase efficiency and reduce energy consumption and greenhouse gas emissions (Baltazar et al., 2021). Indeed, implementing more sustainable options to support plant growth and development, such as the application of biostimulants, and ensuring plant establishment are very important when considering organic and other low-input systems, in which the economic viability of agricultural production has to be achieved under constraints and reduce mineral fertilizers. Biostimulants could also contribute to energy saving in sustainable agriculture, and by doing so, they could help to sustain local employment. (Visconti et al., 2020; Rajabi et al., 2020; Bashir et al., 2021).

The use of biostimulants is becoming more frequent as the management of biotic and abiotic stress that answers current intensive agricultural requirements with sustainable and safe techniques may seek solutions to reduce levels of chemical pesticides through protection products (Cristofano et al., 2021; Del Buono, 2021). For the integrated control strategy, biostimulation and biofertilization will be more often combined with the application of beneficial agents in the future. Such practices constitute an in-depth cultural development of plants through their rhizospheric microbiome and have already shown their

efficacy on diverse crops of economic relevance (Rouphael & Colla, 2020; Castiglione et al., 2021). The principles of growth stimulation and the currently defined modes of action are significant aspects of evaluating biostimulants.

#### **3. BIOPRODUCTS IN ACTION**

Bioproducts are a powerful but deceptively unimposing weapon in the fight to feed the world. Their technology is entirely and uniformly non-toxic. Pioneering innovation in advanced bioproducts commits to forward-thinking product design, which should make us all proud. Biologically based means that a product's core active ingredient is derived from living organisms, including but not limited to quality agricultural chemicals, biofertilizers, and other biostimulants such as growth promoters. Virtually all bioproducts are inherently benign at use concentrations, which makes them the finest tools for safeguarding humans, non-target flora, and fauna alike, as well as our treasured pets and livestock. Modern advanced bioproducts are usually safer than their conventional chemical alternatives. As living agents within much larger organisms, bioproducts focus their benefits on a plant's roots or leaf surfaces rather than dispersing throughout the atmosphere, soil profile, and surrounding watershed (Tolisano & Del Buono, 2023). At present, the contemporary consumer is pressed to unravel the marketing-based consumer definition of 'natural' and determine if any advanced product's claims to 'green' are providing enough science to satisfy expectations regarding non-target issues, efficacy, potential resistance issues, environmental stewardship, and low dramatically diminishing external and unwanted consequences at all levels (Madende & Hayes, 2020; Braun & Colla, 2023; Miranda et al., 2024).

#### 4. ADVANTAGES AND LIMITATIONS OF BIOPRODUCTS

Bioproducts contain natural enemies that are applied to crops to reduce pest populations. This method is compatible with the Integrated Pest Management concept (IPM), which uses all possible combined tactics for control. In addition, natural enemies can complement other chemical and biological control agents, and some bioproducts act synergistically with each other (Baker et al., 2020). Consequently, they can contribute directly to greater integration and synergism in the seed production system by improving the management of the primary soybean pests through the IPM strategy of association, mitigation, and replacement of chemical insecticides (Akutse et al., 2020; Verma et al., 2021; Khursheed et al., 2022). This can contribute to better production practices and reduce the routine use of chemical insecticides in this activity.

The adoption of bioproducts for pest control has been based on the known benefits and virtues demonstrated at a small scale by the natural enemies or hosts that give rise to them. Demonstrating that they do so in the field has been the challenge that has enabled and continues to enable us better to understand biological control agents, including their psychobiology. Due to their relatively high stability, chemical insecticides can protect the crop until it reaches its cycle. However, the amount and number of chemical molecules released cause a reduction in life, speed, release frequency, and even the viability of natural enemies. These factors are aggravated in the case of active ingredients with toxicity to natural pest control agents (Vicente-Díez et al., 2021). For this reason, conventionally, bioproducts, used in association or not, and with or without chemical insecticides, have been applied on a preventative basis before the pest appears, forming a cover that lasts for a certain amount of time. This application prevents the natural enemies, actors of these products, from contacting the chemical compounds (Akutse et al., 2020; Santos et al., 2021).

#### 4.1. Environmental Benefits

Enzymes, antimicrobial peptides, and osmolytes are various and diverse products derived from plants, fungi, or bacteria that hold significant potential to be utilized as innovative biopesticides. Other compelling products, such as medium-chain fatty acids and potassium soaps, could also be regarded as effective biostimulants and biofungicides in agricultural practices. When leveraging an integrated pest management program, the unique characteristics of these substances allow farmers to utilize these biopesticides to suppress pathogen populations, thus safeguarding crop health effectively. Furthermore, farmers participating in collaborative network integration programs can benefit from these biostimulants, which contribute to a considerable reduction in the reliance on conventional agrochemicals and result in enhanced economic benefits that arise from the proper and thoughtful management of agroecosystems (Ayilara et



al., 2023). In the evolving landscape of this century, the generation of new and vital knowledge aimed at tackling the formidable challenges presented by climate change and globalization will lead to profound changes in consumer behavior, prompting necessary adjustments in world trade, which is utterly essential for achieving food security. Such adaptations ultimately empower farmers to produce larger quantities and higher quality food. Research has conclusively demonstrated that food production strategies overly reliant on the increased use of agrochemicals create a pressing dilemma, wherein the quest to produce more ultimately devolves into a relentless race against resilient pests and diseases that continue to gain traction. Furthermore, food production schemes that hinge on chemical pest control do not align with sustainable agriculture principles, which must prioritize maintaining production levels while ensuring that natural resources are used responsibly, safeguarding these resources to ensure that future generations can access vital ecosystem services. The ongoing development of new biopesticides or agrobiotechnological tools aimed at enhancing food security will play a significant role in fulfilling the overarching objectives of sustainable agriculture, mainly due to their low residuality and high specificity in effectively controlling pests. By implementing these innovative solutions, we can achieve a more sustainable future in agriculture while ensuring the health of our planet and its ecosystems (Liu et al., 2021; Fenibo et al., 2022; Silva et al., 2022)

#### 4.2. Comprehensive Residue Management Strategies

Residues of prior crops are raw materials for soil nutrient cycling, representing an enormous storage of plant nutrients. These crop residues should not be considered waste that must be removed from the plant production system by controlled burning, partly because the crust formed on the surface after burning damages the soil structure and the remaining soil organic matter (Sarkar et al., 2020). Total removal of residues will induce nutrient mining and reduce the soil's organic matter content (Shan et al., 2021). However, the return of the crop residues may present disease risks to the following crop, i.e., when residues of a plant with a disease are returned to the soil and contain resting structures or spores that survive on the residue, they will present an infectious source for the next crop. The disease may propagate through the next crop and create problems for the farmer. (Zhao et al., 2020; Fu et al., 2021).



The survival capacity of the pathogens influences the disease pressure, a considerable inoculum potential, and the atmospheric conditions during the growth phase of the crop. When the disaster that is called attention has made its effect, it is evident that the residues should not be mixed with the soil to ensure a biomass layer without the influence of the considerable nutrient content of the residue. Different forms of foliation with fungicidal microbes and plants can be used to control fungal plant diseases in the plant canopy and to develop an extensive residue management strategy (Alghuthaymi et al., 2021). If the diseases were controlled primarily and systemically, pesticide usage could be avoided, and the residues could likely be returned to the soil, as decomposition of the plant tissue and nutrients in the tissue are required for the next crop's growth (El-Saadony et al., 2022; Palmieri et al., 2022).

#### 5. REGULATORY FRAMEWORK FOR BIOPRODUCTS

Bioproducts with direct pesticide effects must be tested for efficacy and safety before being marketed. Current regulatory structures do not adequately cover the range of bioproducts and the degree of variation typical of this category; however, new approaches are urgently needed to ensure that valuable innovations fulfill their potential (Akutse et al., 2020; Priya et al., 2023). Enhancing our understanding of the modes of action and biology of microorganisms and naturally occurring compounds can provide a basis for generating approved claims that do not require uniform product efficacy, often beyond bioproducts' adequate design flexibility. Plant protection in sustainable agriculture can depend on various bioproduct types in markets not served by current chemical-intensive agricultural practices, which rely on disease and weed control by selective herbicides and traditional pesticides (Jain et al., 2022; Otero et al., 2023).

Bioproducts with plant protection and disease control effects are on a fast track for development and commercial use. Some fungi, bacteria, and pheromones have chemical properties that allow them to be governed through the legal and regulatory structures that currently consider and approve traditional pesticides. Other microorganisms and bioactive plant metabolites are prohibited from use as pesticides and fungicides; however, these natural compounds of plants do not fit the receptor, mode of action, and resistance, or often, mandatory percentage of efficacy requirements (Berthon et al., 2021). Despite available technologies



for isolating, identifying, and measuring the concentration of many of these compounds, natural products do not achieve standard, consistent efficacy thresholds when subjected to comprehensive tests on a broad panel of plant species and growing conditions, which would require regulatory approval (El-Saadony et al., 2022; Pacifico et al., 2021).

# 6. FUTURE DIRECTIONS IN BIOPRODUCT RESEARCH AND DEVELOPMENT

There is an abundance of opportunities and ample room for further extensive research on and innovative development of bioproducts specifically tailored for pest management purposes. Numerous fungal entomopathogens and potential bacterial agents could be explored more thoroughly (Bamisile et al., 2021). Still, unfortunately, only a few of these promising agents have been developed for commercial use thus far. The wider adoption and use of biological control agents, in general, have been significantly hampered by the current lack of understanding regarding the behavioral ecology of many pest species and the biological control agents themselves. Several practical approaches could be taken to improve this challenging situation. The increasing incorporation and use of molecular biology techniques to infer relationships among diverse populations of insects will undoubtedly allow for a more detailed and nuanced treatment of insect ecology. Advanced techniques such as DNA probes and even comprehensive DNA sequencing are already finding various applications in the ongoing endeavor to develop commercial biological control products that have the potential to be both practical and environmentally friendly (Islam et al., 2021; Ma et al., 2024)

The discovery of novel bioactive phytochemicals from plants that do not demonstrate phytotoxicity needs to be accelerated to identify new leads for the development of commercial products. Additionally, multidisciplinary teams are required to investigate the potential these bioactive phytochemicals have as part of pest management systems in higher plants and cross-talk signals involved in establishing this resistance (Saroj et al., 2020; Singh et al., 2021). Subsequently, technologies must be developed further to allow commercial producers of bioproducts to scale up and generate adequate production levels to supply agricultural needs. Biotechnology and technological advances in fermentation and formulation offer potential economical solutions to this problem (Lengai et

al., 2020; Souto et al., 2021). The integration of bioproduct pest management systems into heterogeneous environments and sustainable management cropping systems should benefit elite germplasms and may be able to predict the remaining agroecosystem base resistance.

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## CHAPTER 3

## ALGAL BIOREFINERY APPROACH IN BIOTECHNOLOGICAL PRODUCTIONS

Assist. Prof. Dr. Emek ASLAN

Ondokuz Mayıs University, Faculty of Agriculture Department of Agricultural Biotechnology emek.aslan@omu.edu.tr https://orcid.org/0000-0002-1287-3322

#### **1. INTRODUCTION**

In the panorama of biotechnological productions, the potential of algal systems for producing bioenergy or biological materials from sunlight and  $CO_2$  is acquiring growing importance. Algae, thanks to their expansive genetic resource and absence in edible oil production, may represent an effective alternative to terrestrial plants for the extraction of bioactive molecules based on large-scale and so-called cascading uses. Their bioactivity against antimicrobials, antifungals, antioxidants, anti-inflammatory, anti-aging, etc., makes them an unavoidable perspective in the medical market (Kumar et al., 2021). During the lifecycle of algal cultures, the term "biomass" may contain all the organic and inorganic cellular components deriving from growth factors and nutrients integrated into the culture. From the composition and cultivation of algal cultures (species, stress, photobioreactor, etc.), biomass can be used at the end of the cultivation cycle to extract different bioactive compounds. Some remain in the

cell (intracellular), while others emerge outside the cell and can be harvested and exploited (Sun et al., 2022; Wang et al., 2022).

The success of algae system development faces challenges such as largescale selection of algal cultivars to optimize both productivity and product yield, the sustainability of cultivation to reduce carbon footprint, and finally, the adequacy of extraction fractionation processes that are effective, non-polluting, and efficient in terms of cost. The operations of low/high-value recovery using the algal biomass according to the "algae biorefinery approach," especially for innovative technologies, are essential (Slegers et al., 2020; Velvizhi et al., 2022). The biorefinery concept is based on integrating the conversion process into one flowsheet, exploiting the mineral and/or organic fraction of biomass to produce multiple products with high added value in an environmentally friendly and economically advantageous way (Byun & Han, 2020; Rekleitis et al., 2020).

## 2. ALGAL BIOREFINERY: CONCEPT AND PRINCIPLES

The utilization of algae in production holds great promise for exploiting novel bio-resources for advanced biotechnological productions through bio-refinery. Algal bio-refinery is a concept that integrates a variety of bio-processes for the recovery of resources based on algal biomasses. This approach is sustainable and eco-friendly, offering the possibility of contributing to the circular bio-economy by reducing waste and by investing in eco-friendly alternative processes to existing productions of high-added-value compounds. The bio-refinery of algal biomass makes algal production economically viable (Uma et al., 2023; Jain et al., 2024). An example is the extraction of lipids from algal biomass for alimentary use, using the residual extract for biochemical and cosmetic products, and converting proteins, carbohydrates, phenols, etc., produced in the fractionation of the extracted biomass, for pharmaceutical, cosmeceutical, dietetic, and bioremediation uses. The desired final uses lead to the optimal identification of the microalgae appropriate for bio-refinery (i.e., the choice of microalgae with specific metabolism parameters and physiological states such as accumulation of storage components, for example, lipids with a peculiar fatty acid composition). Furthermore, this approach is efficient because of the considerable reduction of waste treatment in agro-industrial processes (Suresh et al., 2023; Venkatraman et al., 2024). Additionally, the pooling of knowledge in

physics, biology, mathematics, and agronomy, focusing on process optimization and control, and a more holistic and integrated vision will be significant.

Algal production depends on converting as much of the light absorbed into the chemical energy that becomes biomass. The energetic efficiency of the algal system is not brutally obtained by dividing the energy value of biomass by the absorbed radiation energy. However, algal production can only be obtained in euros per hectare, and the exploitation of the absorbed radiation energy is possible only if the conversion efficiency of radiation in biomass, which is also given by the radiation use efficiency, is high. The radiation use efficiency translates the efficient conversion of biomass production potential by the mean light flux in an area and is expressed as  $g(MJ^{-1})$ . The energy conversion process and the efficient use of resources are essential for developing this approach in the algal system of bio-production (López-Calcagno et al., 2020; Freitas et al., 2021; Simkin et al., 2022); This vision will be described by defining the steps involved and the technical terms for the algal bio-refinery from the grid's efficiency and energy utilization index in the algal system (energy and material coupling such as CO<sub>2</sub>, water, nitrogen, inorganic salts, and heat), which are crucial to managing the transformation of energy into biomass and facilitating the development of a more interactive energy and material balance in the process. Additionally, to evaluate the biomass algal productivity/post-harvest efficiency, the conversion of nutrients per biomass unit will also be defined (Wang et al., 2021).

## **3. ALGAL BIOMASS PRODUCTION TECHNIQUES**

Producing algal biomass, the basis for many value-added productions, demands cultivation techniques that provide high biomass productivity. Presently, the two main ways of algal biomass production are open pond systems, usually simple, cheap, and potentially extensive but with some constraints, and closed systems, also called photobioreactors, which ensure higher biomass productivity and quality but are more expensive (Xiaogang et al., 2020; Paul et al., 2021). These two main techniques are reviewed in this chapter, as well as some other aspects influencing the choice of the cultivation system: the dependence on algal species, the production chain elements, and other global surroundings like weather conditions, water, and biotic factors. The present research tends towards the optimization and/or correlation and sometimes combination of the different

technical elements involved in the two main techniques (Mahmood et al., 2023).

The open pond—abounding in direct sunlight—is an easily up-scalable and cost-effective cultivation system, which can lead to high productivity but is generally constrained due to contamination problems where the biomass is diluted. At the same time, the advantages of the photobioreactor are its relatively high degree of control compared to the pond system, reduced contamination, high biomass productivity, and a wide range of applications. This text presents the technique therein. It shows some benefits and improvements in the simplified and techno-economically more complex ones, but advantageous. In photobioreactors, the active system, called the closed system, is intensively studied and opens broad perspectives (Ahmad et al., 2021; Benner et al., 2022; Sirohi et al., 2022).

#### 3.1. Open Pond Systems

Open pond systems represent the real incentive for algal biomass production, from which modern biotechnology-based approaches have a substantial foundation. Various tubular, open, and hybrid open pond configurations have been mentioned in science. Typically, the height of these ponds is less, but the length along the ground is around 60-100 m (Saxena et al., 2022). Bolt uniform and raceway configurations are the two main types of open pond systems. The raceway open pond, largely resembling an elongated basin, is found to be the primary location for the requisite culture. Open pond systems can directly exploit natural sunlight, benefiting ecological sustainability by producing a high biomass yield (Gorjian et al., 2022). In consuming large quantities of  $CO_2$  from the environment, these ponds are consistent with the benefit of a clean environment (Priyadharshini et al., 2021).

Open pond systems are primarily large-scale operations due to the lowcost limitations and design restrictions available. One of the most critical shortcomings of these technological approaches is caused by undesirable species in the starting culture and contamination. Nutrient accumulation has been reported due to daily production in open pond system technologies. Therefore, many researchers have improved the product quality and the variation process. Significant difficulties governing open pond processing include low initial capital investment, a large surface area requirement, low biomass concentration, and overall biological containment (Gorjian et al., 2022). Strategies governing the increase in biomass handling focus on two significant aspects: nutrient input and control of photosynthetic rates when biofuel production is maximally attainable. Nutrient supply is the most effective strategy for increasing the biomass yield in open pond systems. Other methods include the elimination of undesirable surrounding microalgae and preventing some wind effects, such as producing more friction in the mixing of cultures (Hoang et al., 2023). As with other production technologies, open pond systems entail investment and maintenance costs. This approach aims to determine whether novel production advantages underlie these expenditures. Open oceans and transitional waters on Earth offer the most significant production potential at the lowest price, enabling global dissemination. Consequently, the open pond system can also draw attention to the advantages of the aforementioned new technologies (Antar et al., 2021; Sarwer et al., 2022).

## 3.2. Photobioreactors

Despite several limitations of pond systems, both indoor and outdoor, necessary advancements have been made in the field of algal biomass production in microscopic to low concentration forms ranging between 0.1-1 g/L. To address such limitations, photobioreactors (PBRs) have been proposed, which provide a controlled environment for algal growth while maximizing the rate of cultivation and biomass production. PBRs facilitate cultivation in temperature-controlled indoor units, ensuring the availability of nutrients, CO<sub>2</sub>, O<sub>2</sub>, cooling, and other factors (El Shenawy et al., 2020; Sathinathan et al., 2023). Moreover, they can also minimize contamination problems by limiting algal density. However, some issues like maintenance and cleaning are associated with large-scale systems. The design of PBRs focuses on the uniform penetration of light in the algal suspension, the exchange of gases, and temperature control (Benner et al., 2022; Peter et al., 2022; Sirohi et al., 2022).

The characteristics of PBRs can be a decisive factor in photoautotrophic production processes. In tubular photobioreactors, energy, and  $CO_2$  transfer can be compromised in the aerobic cultivation of bacteria, fungi, or yeast. For instance, small tubular photobioreactors are generally used for the low-volume production of microalgae, high-value molecules, and non-photosynthetic



organisms or for storing and concentrating cultures. Several companies promote different models of cost-effective tubular photobioreactor systems, using a mix of hard and soft tubes to increase stability and resistance while decreasing costs. Flat-panel photobioreactors (PBRs) have been demonstrated to facilitate ease of cleaning and operation in large parallel systems (Sirohi et al., 2022; Sathinathan et al., 2023). Numerous commercial entities offer one- or two-panel PBRs to cultivate various algal species. A cost-benefit analysis of these systems has revealed no discernible trade-off between investing in cutting-edge hardware and achieving higher yields in large-scale systems. The assessment has also encompassed the selection of culture species that do not necessitate elevated light levels or the development of adaptive control to synthetic light, ensuring more efficient algal cultivation and reducing costs associated with microalgae production (Assunção & Malcata, 2020; Liu et al., 2022). The main argument of many works providing economic evaluations of photobioreactors relates to the capital investment required to create photosynthesis-based algal cultivation. Economically, the photobioreactor is an energy-intensive asset and requires aeration to provide the best cultivation conditions (El Far et al., 2021). However, there are possibilities to enhance cultivation under controlled conditions and operate safely. Innovations are currently present in the literature that can enhance algal cultivation on a large scale or under controlled conditions, further reducing the cost of products that can easily reach different markets. (Fabris et al.2020; García-Poza et al., 2020; Yong et al., 2021)

#### 4. ALGAL BIOREFINERY PROCESSES

Harvesting of algal biomass serves as the primary task in algal bio-refinery, followed by initial dewatering of the harvested algal biomass at a larger scale, which may affect the total economic viability of the biotechnological process. Mechanical methods or cultivation process designs can harvest algal biomass. Harvesting is generally followed by algal biomass dewatering, commonly known as pre-dewatering methods, to eliminate extra cell-associated water. Following harvesting and initial dewatering, downstream processes convert dry or wet algal biomass into the desired final quality products using appropriate aqua-solvents, especially by using acceptable technical solvents to leach out the target compounds from algal biomass (Goswami et al., 2022). Algal active

compounds are used as a growth bio-stimulant, and these natural biotechnological products possess highly commercial values. After extraction of these bioactive compounds, the remaining waste of algal cells is utilized as algal bilge or dregs to combine back for the commercial installation of algal biorefineries or to be used for animal feeds after pre-treatment, methane solvation, or as bio-compost material. (Javed et al., 2022; Thanigaivel et al., 2022; Zhang et al., 2023)

Biofuel conversion from algal biomass is crucial in determining its economic viability in commercial and industrial-scale algal biorefineries. It is a fact that algal cells are rich in oils like triglycerides and polyunsaturated fats, which can be converted into algal fuel for commercial purposes. Hence, fourth-generation conceptual algal bio-refinery is focused on producing algal fuels using different biotechnological, thermochemical, and biochemical engineering pathways and a multi-algae species band for bio-refinery processes (Thanigaivel et al., 2022). Thereafter, the solvents used for active compound leachate are used again for biodiesel production by trans-esterification or other chemical engineering steps to recruit algal solvent fuels (Saravanan et al., 2023; Banerjee et al., 2022). In the algal bio-refinery process, there is an interrelationship between all algal processes so that researchers working on algal processes can understand and study each process to optimize and utilize algal biomass to extract maximum biooil, biodiesel, bio-gases, and other bio-products for economic and sustainability grounds (Saral et al., 2022). Maintaining these processes commercially and industrially through the environment is an innovative process. Post algal cultivation and harvesting; the next critical step is the biomass pre-treatment and extraction process of the active compounds required by various industries worldwide. Recently, the algal biomasses and associated biotechnological processes adopted by investigators used various innovative technologies and methodologies to upgrade them for a commercial and industrial scale fitting to attain the best prudent course (Thanigaivel et al., 2022; Kashyap et al., 2023).

## 4.1. Biomass Harvesting and Dewatering

Biomass harvesting, which precedes conversion, includes separating algal cells from the growing medium. However, a simple separating approach does not apply to algal culture since algae are often present as a fine suspension. Harvesting and dewatering are traditionally two separate stages in algal bio-



refinery. It should be noted that effective biomass separation to acquire a liquidphase growth medium is as essential as cell harvesting in the bio-refinery process, in contrast to bio-conversion (Kostas et al., 2021; Zhang et al., 2023). The market supply of large-scale algal dewatering is minimal compared with the development of harvesting technologies. Dewatering is frequently linked with drying since it is the essential cost of encapsulation and containment of the algal product. Thus, developing an efficient and cost-effective process of algal biomass dewatering is vital (Thanigaivel et al., 2022; Kumar et al., 2022)

Biomass harvesting facilities in full-scale algal cultivation consist of three steps: water removal, residue extraction from the cultivation medium, and dewatering this residue to produce a functional product. Thus, the algal culture broth must undergo several processing steps to obtain a solid pad containing greater than 95% (w/w) of algae. Available techniques for harvesting algal biomass can be divided into four categories: sedimentation and flotation, centrifugation, filtration, and current harvesting methods. There are multiple techniques for harvesting. The maximum recovery of valuable products from the algal biomass is always needed with minimal energy consumption (Ortiz et al., 2021). Conceptually, it is recognized that gravity-based separation systems (sedimentation, sieving, flotation) are cheap and use less energy than other methods; however, they are not feasible for delicate suspensions due to the slowness of their separation rates. The apparent density of microalgae and water is the key to their harvest since it allows gravity-based harvesting (Tan et al., 2020). In these gravitational methods, there are two steps: up-concentration of the algae and solid-liquid separation. Thus, these downsides are addressed in other methods: enhanced gravity, leading to faster separation, and circular or rotatory flows to separate the different phases (Tan et al., 2020; Mohan et al., 2021).

#### 4.2. Extraction of Bioactive Compounds

The demand for bioactive compounds derived from algal biomass has prompted the development of suitable extraction techniques. Extraction methods such as solvent extraction, supercritical fluid extraction, and microwave-assisted extraction have been proposed and analyzed (Uma et al., 2023). The selection of an extraction method is contingent upon the type of bioactive compound of interest, the efficiency of the method, its cost, and its environmental impact. The extraction of carotenoids, a major group of pigments, occurs during solubilization in an oily phase due to their lipophilic nature or other water-soluble pigments in a micellar phase (Yang et al., 2023). Lipids can be extracted using mechanical methods or, more efficiently, liquid-liquid extraction or other methods. The third group of bioactive compounds extracted from algae is carbohydrates, which are extracted, for example, using supercritical fluid extraction and ionic liquids (Feng et al., 2022). The aforementioned compounds are extracted for their potential pharmaceutical applications. Algal extracts can also be used in the cosmetics, food, and animal feed industries (Menaa et al., 2021; Olguín et al., 2022; Patel et al., 2022).

The general framework for efficiently extracting valuable compounds from algal biomass, as a crucial part of algal biorefinery, is outlined. Market predictions indicate that the bioactive compounds present in microalgae and macroalgae attract researchers from the field of biotechnology. However, the extraction of valuable compounds from algae is a mammoth task. There are several difficulties during the extraction of a valuable compound from algae, including (1) stable algal compounds, for example, the lipids present in the green algal molecules of *Chlorella vulgaris*, and (2) cost efficiency of the extraction process, which may not produce enough yield from microalgae (El Far et al., 2021; Foo et al., 2021; Nieri et al., 2023).

## 4.3. Biofuel Production

The conversion of algal biomass to energy removes excess carbon from the atmosphere and offsets the burning of fossil fuels. Many methods are available that are used to convert lipids in the form of algal biomass into renewable bioenergy, which is produced by transferring many pathways of algae into biofuels: hydrocarbons, triglycerides, other lipids, and carbohydrates. These have long been used to produce biofuels in the United States, Europe, and other regions. These include biodiesel obtained from trans-esterifying microalgae oils and bioethanol produced using top-down methods, commonly used in reducing  $CO_2$  emissions and curbing global warming (Ganesan et al., 2020; Sarwer et al., 2022). However, the path leading to the production of such liquids is not economical. For economic competitiveness, many internal steps in the processing



chain are required for new methods or advanced production processes. This would help with worldwide environmental issues since CO<sub>2</sub> emissions are rising due to the burning of fossil fuels. Production from microalgae of algal biofuels has also improved, producing up to 136,000 gallons of biodiesel per 1.225 acres per year. Successful examples of algal biofuel approaches include petroleumbased biofuels, sources of exhaustible energy that produce greenhouse gases and other pollutants. An advantage of biofuels obtained from microalgae, in particular unsaturated molecules, over other microorganisms is their rapid growth, colossal CO, absorption, and a low land space requirement for a large lipid product of all wet biomass compared to plants, as well as their non-use of fresh water for large-scale cultivation (Tang et al., 2020; Khoo et al., 2023). Categories of mass-produced species may be used for biodiesel, bioethanol, and methane. The increased CO<sub>2</sub> market is one of the main factors in the success of algal biofuel production. Therefore, the market should be both environmentally and economically attractive, with specific laws and directives from the fuel industry on the quality of algal biodiesel (Khan et al., 2024).

# 5. FUTURE PERSPECTIVE FOR ALGAL BIOREFINERY IN BIOTECHNOLOGICAL PRODUCTIONS

In recent years, many scientists have devoted increasing efforts to the innovative biotechnological production of various microalgae species. Extensive research has demonstrated a rising demand for biomass extracted from different microalgae varieties in numerous industrial sectors, including food, animal feed, and oleochemical applications (Tan et al., 2020; Yap et al., 2021). Given this surge in demand, it becomes crucial to develop and implement new approaches that enable full and effective utilization of algae. This leads to the capability of producing essential substances not just in more significant quantities but, whenever possible, from the same biological matter, which is being exploited more intensively now than ever before (Muhammad et al., 2021; Show, 2022; Siddiki et al., 2022). This approach provides various aspects and insightful details regarding the biorefinery concept, specifically within the algal field, with a scope of significant biotechnological productions derived from microalgae and their applications.

The employment of renewable raw materials and the exploitation of efficient



bioconversion techniques are crucial elements that must be considered in the current scenario, which is characterized by an ever-growing energy demand. This demand is coupled with a series of major global concerns that encompass the climatic problems faced by our planet, the harmful effects of greenhouse gases that are directly related to the combustion of fossil fuels, the increasing scarcity and depletion of conventional natural resources, as well as the environmental and ethical considerations that cast long shadows over biofuel production based on crops (Duque et al., 2021; Moshood et al., 2021; Lee et al., 2022) Although the above considerations are frequently referred to specific bioenergy applications, it is essential to note that burnable biogas, bioethanol, and biodiesel are not the only obtainable products that can be derived from selected raw renewable materials through specific biotechnological processes (Ahmed et al., 2023). In a similar manner and numerous instances, the strategic focus of several member states has enabled the procurement and articulation of various substances that possess substantial potential not only for bioenergy but also for other industrial domains and research endeavors.

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## **CHAPTER 4**

# EFFECT OF COLLAGEN ON COLOR YIELD IN NATURAL DYEING

#### **Eray ARSLAN**

Textile and Manuscripts Conservation Restoration R&D Laboratory Researcher

## Emircan GÜNGÖR

Marmara University, Faculty of Technology, Department of Textile Engineering, Istanbul, Turkey

## Meral ÖZOMAY

<sup>3</sup> Marmara University, Institute of Neurological Sciences, Istanbul, Turkey meralozomay@gmail.com

Semra ÜNAL Marmara University, Institute of Neurological Sciences, Istanbul, Turkey.

#### **Shahid ADEEL**

Department of Applied Chemistry, Government College University Faisalabad, Faisalabad, Pakistan

#### Zafer ÖZOMAY

Marmara University, Faculty of Applied Sciences, Department of Printing Technologies, Istanbul, Turkey



#### INTRODUCTION

The mordanting process is necessary to bind natural dyes to textile fibers and enhance their fastness properties. However, some mordant agents, particularly metal salts used in these processes, have been frequently criticized worldwide from an ecological perspective and have become a significant problem in natural dyeing [1-2]. Mordants are mineral salts that help the dye penetrate the fabric structure and adhere firmly to the fibers. Substances such as alum (potassium aluminum sulfate), tin (tin chloride), chromium (potassium dichromate), ferrous sulfate, and copper sulfate (blue vitriol) are among the primary mordanting agents used with natural fabric dyes [3-5]. Although these metal mordants contribute to the fixation of the dye and the formation of a broad color spectrum by forming complexes with natural coloring compounds, many of these metals are inherently toxic, and it can only be said that their minimal presence poses relatively less harm to users [6-8]. Replacing environmentally hazardous synthetic mordants with natural mordants is a significant challenge that needs to be addressed [9-13]. Recent studies have shown that collagen can be used in textile dyeing [14-16].

Furthermore, Type I collagen derived from waste animal bones has been discovered as a new application in textile finishing processes. Research in the literature indicates that collagen significantly improves the flame retardancy and mechanical properties of textile materials. The use of collagen in cotton, Tencel, and Tencel/cotton blend fabric samples has resulted in a significant increase in wet rubbing fastness values [14].

Collagen is a family of proteins composed of at least 29 different types found in various forms such as fibrous, non-fibrous, fibril, microfibril, and membrane [17]. Type I collagen is the most common type of collagen in the human body and is found in the skin, bones, tendons, and connective tissues. Type II collagen is located in cartilage tissue, while Type III collagen varies with age, and other collagen types are present in small amounts depending on the organ [17-18]. Each type of collagen has a unique amino acid sequence and molecular structure, distinguishing them from one another. The amino acid sequences in collagen are responsible for functions such as energy storage, stiffness, or elasticity [17]. Collagen consists of 19 different amino acids, with glycine, proline, and hydroxyproline accounting for approximately 57% of the amino acids in



collagen. Like other proteins, collagen has primary, secondary, and tertiary structures. Three different primary polypeptide chains come together to form tropocollagen, which is the fundamental building block of collagen [17, 19].

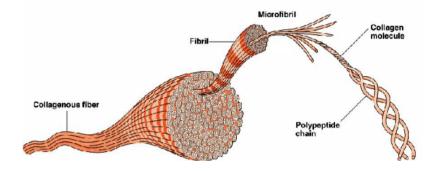


Figure 1. Collagen Structure

Collagen is categorized into seven different classes based on its molecular structures, with a total of 19 different types identified, each performing specific functions in various tissues. Type I, II, III, V, and XI collagens are classified as fibrillar collagens due to their fibrous structures, while other collagen types are typically described as non-fibrillar collagens due to their network or sheet-like structures [20-22].

Type I and Type II collagens, the most common types found in connective tissue and used in the food industry, play critical roles in providing resilience to the skin. Scientific studies have revealed that collagen demonstrates antioxidant properties upon absorption and subsequently exhibits biological activities. Collagen supplements contain bioactive components that enhance moisture content, prevent skin aging, and contribute to the regeneration of joints and connective tissues. Some of the Applications of Collagen in Various Industries [23-24].

#### Structural and Protective Functions;

Collagen forms the extracellular matrix, providing structure and support to tissues. It imparts elasticity to bones, cartilage, tendons, and skin, contributing to the development of tissues and organs. Collagen acts as a barrier, protecting the skin from toxins and pathogens [25]. It aids in the healing and repair of damaged bones and blood vessels. Due to its excellent water retention, skin-repairing



properties, and low allergenicity, collagen is widely used in the cosmetic industry [26]. A reduction in glycosaminoglycan (GAG) and hyaluronic acid levels in the skin is also crucial in skin aging.

Marine-derived collagen has potential applications in sunscreens, shampoos, hair gels, nail polish, and lipsticks [27]. Additionally, marine collagens are commonly used in cosmetic products as nail strengtheners and hair nourishment agents [28].

## Pharmaceutical Industry;

Collagen is extensively utilized in pharmaceutical and biomedical fields due to its low antigenicity, cell adhesion ability, biodegradability, and biocompatibility [29-30]. It is used in micro-particles, injectable mixtures, and drug delivery systems.

## Tissue Engineering;

Tissue engineering focuses on using cells and biological materials to repair or regenerate damaged or diseased tissues. Collagen types I, II, III, V, and XI, containing fibrils, form the basis of biomaterials in this field. Collagen's high biocompatibility and similarity to natural tissue make it an ideal material for tissue engineering [31,32]. Type I collagen, regarded as the gold standard, is used as a scaffold in cell culture systems, enabling cells to grow and develop in a natural-like environment. Collagen-based biomaterials find extensive applications in injectable matrices, scaffolds for bone regeneration, and other tissue engineering uses.

## Biomedical Industry;

Collagen plays a crucial role in the regeneration and repair of tissues, one of the most complex areas in modern medicine. It serves as a natural scaffold system, supporting the growth and development of new cells. Extracellular matrix (ECM) collagen, derived from porcine or human dermis or tissues like intestinal submucosa, is widely used in tissue engineering and regenerative medicine applications. Collagen scaffolds are also utilized for visualizing cells in nervous system models [31-33].

## Medical Applications;

Collagen-based scaffolds are pivotal in modern medicine, aiding in the repair and regeneration of tissues damaged by injury or disease, significantly improving quality and longevity of life [34]. These scaffolds support the natural repair of cartilage and bone tissues, playing a vital role in treating arthritis, osteoporosis, and sports injuries. Collagen films, powders, and sponges used in surgical sutures, wounds, and burns expedite healing and reduce infection risks. Applications extend across areas such as cornea defects, neural migration, dental and bone grafts, and even obesity and arthritis treatments [35]. Collagen also has groundbreaking applications in cardiology, dermatology, orthopedics, ophthalmology, urology, and vascular medicine [36].

## Textile Industry;

Type I collagen derived from waste animal bones has been discovered as a new application in textile finishing processes. Studies indicate that collagen significantly enhances the flame retardancy and mechanical properties of textile materials. Its use in cotton, Tencel, and Tencel/cotton blend fabric samples has resulted in a substantial increase in wet rubbing fastness values [14, 37-39].

In recent years, the importance of natural dyeing has increased. The reasons are as follows:

- The wide applicability and significant potential of natural dyes.
- Experimental evidence showing that natural dyes lack the allergic and toxic effects associated with some synthetic dyes.
- The potential to create employment and revive traditional dyeing technologies, which serve as a livelihood for artisans.
- Stimulating non-food agriculture required for the production of natural dyes and creating employment opportunities in this field [40-47].

Onion, botanically known as Allium cepa L., belongs to the Liliaceae family. The plant originates from the Middle East and is widely cultivated in regions such as India, South India, and Bengal [43]. The onion plant is a perennial that grows up to approximately 1.2 meters tall, with 4-6 hollow cylindrical leaves. The underground bulb of the onion plant consists of fleshy, leaf-like sheaths



that form a thin-skinned capsule. Depending on the variety, the bulb exhibits significant differences in size (2-20 cm), shape (flattened, spherical, or pear-shaped), and color.

The primary coloring component found in the dry outer skin of the onion is quercetin (C15H10O7), a flavonoid, along with proto-catechuic acid, kaempferol, anthocyanidin, and some tannins. The outer layer of the onion, accounting for 10-25% of its total weight, is removed before use and is considered a waste product in the food industry. With India being the second-largest producer of onions globally, yielding 22.43 million tons, a substantial amount of onion peel waste is generated [48].

Onion peels derived from onion waste contain flavonoids, flavonols, antioxidants, and other phytochemicals. Flavonols such as quercetin and its derivatives play a role in producing various brown and yellow colors, while anthocyanins contribute to the purple and red hues in other varieties [49].

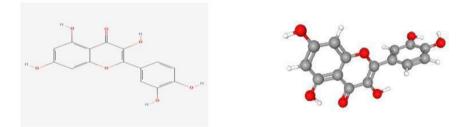


Figure 3. 2D and 3D representations of Quercetin [50].

Cochineal is an important insect used in obtaining natural dyes, found on cacti of the Opuntia and Nopalea species, particularly in regions with Nopalea cochenillifera in Middle and tropical South America [51]. The natural dye is extracted from the female body of the cochineal insect (Dactylopius coccus costa). The coloring agent, known as carminic acid, is widely used to color foods, cosmetics, beverages, pharmaceuticals, and textiles. Dyes obtained from cochineal insects are among the largest natural sources of red-purple dyes. The primary pigment (carminic acid) is mostly extracted from cochineal insects that inhabit cactus hosts [52].

Female cochineal insects reach egg-laying maturity within 90-110 days. Approximately 100,000 cochineal insects collected during the 90-110 day period, when the highest pigment concentration is achieved, yield 1 kg of raw



cochineal. The collected cochineal insects can be dried using various methods, such as immersion in boiling water, oven drying, or sun drying. Among these methods, sun drying is the most efficient and produces the highest quality natural dye. To preserve the insects without degradation, they must be dried to about 30% of their initial weight [51].

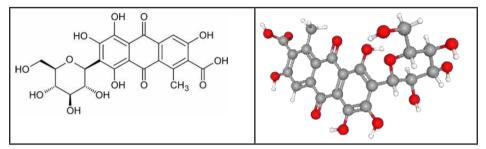


Figure 4. Chemical structure and three-dimensional appearance of carminic acid [53]

## MATERIAL AND METHODS

## Material

In this study, 100% cotton and 100% silk fabrics were dyed with natural dyes obtained from cochineal and onion peel. Collagen was used as mordant material.

## Method

In this study, Type I collagen was used as a natural mordant through a premordanting method. The study utilized onion peel waste as a plant-based natural dye, cochineal insect as an animal-based natural dye, cotton fabric as a plantbased material, and silk fabric as an animal-based material. Color differences were compared separately in relation to dyeing without a mordant, as well as between plant-based and animal-based fibers and dyes. The results were used to comparatively analyze the effects of collagen on the dyeing of plant-based and animal-based fibers.



#### **Preparation of natural dye extracts**

To compare the effect of collagen in animal-based and plant-based dyeing processes, cochineal was used as the animal-derived dye, while onion skins, classified as food waste, were used as the plant-based dye. The onion skins, obtained from onion processing companies, were dried at ambient temperature and ground using a grinder before use. Similarly, dry cochineal insects were also ground with a grinder before use.

To obtain the extracts, the dye materials Allium cepa L. (onion skins) and Dactylopius coccus Costa (cochineal insects) were separately weighed and soaked in distilled water at 18–22°C for 24 hours. After 24 hours, the mixtures were heated on a hotplate and brought to boiling temperature within 10 minutes. The boiling process was carried out in distilled water at 100°C on a hotplate for 60 minutes. During boiling, the evaporated water was checked every 15 minutes and replenished as needed. After boiling, the obtained extract was left to rest in the bath for 24 hours and then filtered for use.

## Mordanting stage

The pre-mordanting method was used. To compare the binding effect of collagen on animal and plant-based fibers during dyeing, the animal-based fiber silk and the plant-based fiber cotton were separately mordanted. After dyeing, the effect of collagen was evaluated comparatively.

Pre-Mordant Recipe

- 5g/L 10g/L 20g/L 30g/L 40gL Collagen
- 45 °C
- 60 minutes
- pH 4.5
- 1/50 Flotte
- Dry Without Rinsing

## Dyeing of Fabrics

100% silk and 100% cotton fabrics were dyed with collagen in combination with the plant-based dye (onion skins) and the animal-based dye (cochineal).



Color differences were compared separately, both in relation to dyeing without mordanting and between plant-based and animal-based fibers as well as plantbased and animal-based dyes. Based on this comparison, the effects of collagen on the dyeing of plant-based and animal-based fibers were examined comparatively. Dyeing Fabrics That Have Been Mordanted

Onion Peel Dyeing Recipe

- 100% dyestuff
- 100°C
- 60 minutes
- 1/50 Flotte
- Wait 3 hours, rinse and dry
- pH 4.5

**Cochineal Dyeing Recipe** 

- 10% dyestuff
- 100°C
- 60 minutes
- 1/50 Flotte
- Wait 3 hours, rinse and dry
- pH 4.5

## FASTNESS TESTS

After the dyeing process, the fabric samples were subjected to tests for color measurement, rubbing fastness, light fastness, and wash fastness.

Color measurements were taken at three different points on the fabric using a Konica Minolta CM-700d color spectrophotometer. The L\*, a\*, b\*, and SCI data were recorded, and K/S (color yield) values were calculated.

The ISO 105 X12 standard was applied to the fabric samples using a T600SH Manual Crockmeter for rubbing fastness testing. Two types of rubbing tests, dry and wet, were performed, and the staining levels were determined using a gray



scale. In the evaluation, a score of 1 indicated the worst performance, while 5 indicated the best.

The light fastness test was carried out using a Solarbox 1500E device following the ISO 105 B02 standard. The degree of fading was assessed under a light cabinet using a blue scale, with scores ranging from 1 (worst) to 8 (best).

Wash fastness testing was completed using a Gyrowash machine following the ISO 105 - C06 standard. After the fabrics were removed from the device and dried, staining and fading levels were assessed using a gray scale under D65 light. The evaluation ranged from 1 (worst) to 5 (best).

## FINDINGS

The given sample recipes and the effect of different collagen amounts on color yield were examined based on these recipes.

 $L^* = Lightness$ 

 $a^* = \text{Red} / \text{Green coordinate } +a^* \text{ red}, -a^* \text{ green}.$ 

b\*= Yellow / Blue coordinate +b\* yellow, -b\* blue.

K and S = Indicates the absorption and scattering coefficients of the film layer.

SCI = Indicates the measurement including total reflection.

$$K/_{S} = \frac{1 - SCI^{2}}{2 * (\frac{SCI}{100})}$$



**Table 1.** Colour values obtained by dyeing cotton fabrics with onion peels,

 which have been previously mordanted

Materiel	Mor- danting Method	Collagen Amount (g/L)	L*	a*	b*	SCI	K/S
Onion Peel	Without Mordant		80.41	5.05	15.83	74.21	0.044813644
Onion Peel	Pre.	5g/L	79.49	6.07	16.55	74.22	0.044772864
Onion Peel	Pre.	10g/L	77.51	6.98	16.47	73.18	0.049146789
Onion Peel	Pre.	20g/L	78.57	6.98	17.69	74.24	0.044691379
Onion Peel	Pre.	30g/L	77.88	7.28	16.56	73.74	0.046758042
Onion Peel	Pre.	40g/L	79.02	6.65	18.34	74.63	0.043121861

When cotton fabrics dyed with onion skins through pre-mordanting processes were examined, it was found that the addition of 10 g/L and 30 g/L pre-mordant resulted in darker colors.

**Table 2.** Color values obtained from dyeing silk fabrics with onion skins after

 pre-mordanting processes.

Materiel	Mordan- ting Met- hod	Collagen Amount (g/L)	L*	a*	b*	SCI	K/S
Onion Peel	Without Mordant		69.59	13.07	21.26	66.64	0.08350012
Onion Peel	Pre.	5g/L	68.7	11.58	21.21	63.88	0.102117595
Onion Peel	Pre.	10g/L	68.44	12.86	22.03	65.55	0.090526506
Onion Peel	Pre.	20g/L	70.24	12.24	19.65	66.07	0.087123119
Onion Peel	Pre.	30g/L	69.87	12.82	21	66.44	0.0847587
Onion Peel	Pre.	40g/L	71.32	11.66	20.15	66.86	0.082131289

When the processes subjected to pre-mordanting were examined, the addition of 10g/L and 40g/L pre-mordant positively increased the color yield in the fabric.



**Table 3.** Colour values obtained as a result of dyeing cotton fabrics with cochineal which have been previously mordanted.

Materiel	Mordan- ting Met- hod	Collagen Amount (g/L)	L*	a*	b*	SCI	K/S
Cochineal	Without Mordant		80.11	7.65	5.78	74.59	0.043281144
Cochineal	Pre.	5g/L	81.91	7.39	5.01	82.21	0.019248516
Cochineal	Pre.	10g/L	81.81	6.11	5.07	82.39	0.018819766
Cochineal	Pre.	20g/L	82.77	6.2	4.11	82.9	0.017636309
Cochineal	Pre.	30g/L	83.2	7.98	6.24	83.03	0.017341979
Cochineal	Pre.	40g/L	82.62	7.55	5.62	83.18	0.017006035

As a result of the application, it was observed that the color yield decreased in cotton materials dyed with cochineal dye. Although the color yield values obtained at different concentrations of the mordant in the mordanting method showed similar results, a decrease in color yield was particularly evident in premordanted applications compared to non-mordanted fabrics.

**Table 4.** Colour values obtained by dyeing previously mordanted silk fabrics

 with cochineal

Materiel	Mordan- ting Met- hod	Collagen Amount (g/L)	L*	a*	b*	SCI	K/S
Cochineal	Without Mordant		58.55	25.92	14.8	66.97	0.081452957
Cochineal	Pre.	5g/L	62.51	20.15	8.34	69.54	0.066710641
Cochineal	Pre.	10g/L	61.01	23.48	13.63	69.91	0.064755264
Cochineal	Pre.	20g/L	61.72	24.99	14.42	75.91	0.038224746
Cochineal	Pre.	30g/L	61.45	24.96	14.76	76.91	0.034660519
Cochineal	Pre.	40g/L	63.48	23.74	11.82	78.36	0.029880653

When the processes applied to silk fabrics were examined, it was observed



that the non-mordanted fabric in the pre-mordanting process yielded better color efficiency. It was also found that smaller amounts of mordant resulted in better color yield compared to higher concentrations of mordant.

**Table 5.** Fastness values obtained as a result of dyeing cotton textile material

 with onion peel using the pre-mordanting method

Cotton-O	nion					Rubbing Fastness				
Mordant (g/L)	Light Fastness	Color Change	Stain	ing Fas		Dry	Wet			
			WO	PAN	PES	PA	со	CA		
Without Mordant	2-3	2	5	3	5	5	5	5	5	4-5
5g/L	2	2	5	5	5	4	4	5	5	4-5
10g/L	2	2	5	5	5	4	4	5	5	4-5
20g/L	2	2-3	5	5	5	5	4	5	5	4-5
30g/L	2	3	5	5	5	4	4	5	5	4-5
40g/L	3	3	5	5	5	5	4	5	5	4-5

When examined in the table, it was observed that the addition of mordant did not cause a significant change in dry and wet rubbing fastnesses and staining and fading fastnesses.

Silk -Onio	n	Washing	fastne	SS					Rubb Fastn	
Mordant (g/L)	Light Fast- ness	Color Change	Stain	ing Fast	1	Dry	Wet			
			WO	PAN	PES	PA	со	CA		
Without Mordant	2	3	5	4	5	5	5	5	5	5
5g/L	4	5	5	5	5	4	4	5	5	5
10g/L	2	4	5	5	5	4	5	5	5	5
20g/L	2	3	5	5	5	4	4	5	5	5
30g/L	3-4	4	5	5	5	4	4	5	5	5
40g/L	2-3	5	5	5	5	4	4	5	5	5

**Table 6.** Fastness values obtained as a result of dyeing silk fabric with onion

 using the pre-mordanting method

Silk fabrics were dyed with onion skin in combination with the pre-mordanting process. When examining the fastness results, variable values were observed in the light fastness results. It was found that the addition of 5g/L and 30g/L pre-mordants resulted in light fastness results similar to each other and improved compared to the unmordanted dyeing, while the addition of 40g/L mordant showed a relatively better light fastness, but the addition of 10g/L and 20g/L pre-mordants did not lead to any change in the light fastness compared to the unmordanted fabric. Generally, an improvement in fading fastness values was observed, with only the 20g/L pre-mordanting process showing the same fading fastness value as the unmordanted fabric. In the 5g/L and 40g/L pre-mordanting processes, very good fading fastness values were achieved after fabric washing. When examining the fiber staining after washing, it was found that both premordanted and unmordanted dyeing resulted in similar results. No changes were observed in the wet and dry rubbing fastness values in both mordanted and unmordanted methods, and good fastness results were observed.

**Table 7.** Fastness values obtained as a result of dyeing silk fabric with cochineal using the pre-mordanting method

Silk - Coc	hineal	Washing	fastne	<b>S</b> S					Rubb Fastn	
Mordant (g/L)	Light Fast- ness	Color Change	Stain	ing Fast		Dry	Wet			
			WO	PAN	PES	PA	со	CA		
Without Mordant	4	1	5	5	5	5	5	5	5	5
5g/L	3	1	5	5	5	5	5	5	5	5
10g/L	3	1	5	5	5	5	5	5	5	5
20g/L	3	1	5	5	5	5	5	5	5	5
30g/L	3-4	1	5	5	5	5	5	5	5	4-5
40g/L	3	1	5	5	5	5	5	5	5	4-5

When examined in the table, it was observed that the addition of mordant did not cause a significant change in dry and wet rubbing fastnesses and staining and fading fastnesses.

**Table 8.** Fastness values obtained as a result of dyeing cotton fabric with cochineal using the pre-mordanting method

Cotton - C	Cochineal	Washing fastness								Rubbing Fastness	
Mordant (g/L)	Light Fast- ness	Color Change	Stain	ing Fast		Dry	Wet				
			WO	PAN	PES	PA	со	CA			
Without Mordant	2-3	1	5	5	5	5	5	5	5	5	
5g/L	3-4	1	5	5	5	5	5	5	5	5	
10g/L	4-5	1	5	5	5	5	5	5	5	5	
20g/L	4-5	2	5	5	5	5	5	5	5	5	
30g/L	3-4	2	5	5	5	5	5	5	5	5	
40g/L	4-5	1	5	5	5	5	5	5	5	5	



In the samples dyed with the pre-mordanting method, no change was observed in the rubbing fastness compared to the unmordanted fabric, and both mordanted and unmordanted fabrics showed good wet and dry rubbing fastness. In the premordanting processes, however, it was observed that the fading fastness values were poor after washing, while the staining fastness values were good. The light fastness results, regardless of the mordanting method, showed different fastness values depending on the concentrations. It was observed that the addition of 10g/L, 20g/L, and 40g/L mordants improved the light fastness values compared to the unmordanted fabric.

#### CONCLUSION

In this study, the effect of collagen as a mordant in natural dyeing on color yield and its impact on fastness properties were investigated. No significant difference in color yield was observed when silk and cotton fabrics were dyed with cochineal dye using pre-mordanting processes. However, when onion skin, a natural dye, was used, a contrasting situation was observed in cotton fabrics compared to cochineal-dyed fabrics. In the pre-mordanting process, cotton fabrics dyed with 10g/L and 30g/L mordant solutions showed higher color yield compared to unmordanted fabrics. In silk fabrics, the pre-mordanting process with 10g/L mordant solutions increased color yield.

When the fastness values were examined, it was found that cotton fabrics dyed with onion skin showed better light fastness and washing fastness values as the concentration of the mordant increased. Additionally, a slight improvement in wet rubbing fastness values was also observed.

As a result, based on the data obtained from this study, where Type-I animal collagen was used directly, the positive effect of collagen on color yield and fastness properties was confirmed. The results suggest that experimental studies can be conducted with different types of collagen.



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# **CHAPTER 5**

# COMPARISON OF COLOR MEASUREMENTS AND FASTNESS VALUES OF BACTERIAL CELLULOSE DYED WITH CONVENTIONAL METHOD

## Emircan GÜNGÖR

Marmara University, Faculty of Technology, Department of Textile Engineering, Istanbul, Turkey

**Eray ARSLAN** 

Textile and Manuscripts Conservation Restoration R&D Laboratory Researcher

## Meral ÖZOMAY

<sup>3</sup> Marmara University, Institute of Neurological Sciences, Istanbul, Turkey meralozomay@gmail.com

Semra ÜNAL Marmara University, Institute of Neurological Sciences, Istanbul, Turkey.

#### **Shahid ADEEL**

Department of Applied Chemistry, Government College University Faisalabad, Faisalabad, Pakistan

## Zafer ÖZOMAY

Marmara University, Faculty of Applied Sciences, Department of Printing Technologies, Istanbul, Turkey



## **INTRODUCTION**

Cellulose was discovered by Payen in 1838 [1]. It is one of the most abundant biopolymers in nature and constitutes the cell walls of plants. Structurally, cellulose is an unbranched polymer formed by the  $\beta$ -1,4 linkage of glucopyranose units. Bacterial cellulose, on the other hand, is synthesized by microorganisms and consists of linear glucan molecules bonded by hydrogen bonds, making it a multifunctional nanobiomaterial. It appears similar to plant cellulose [2].

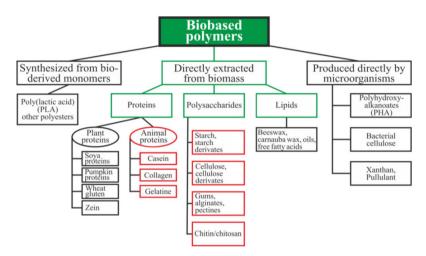
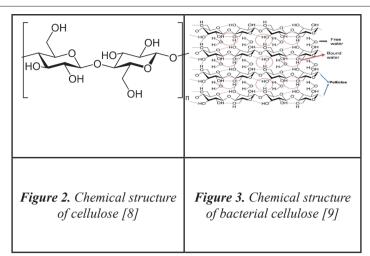


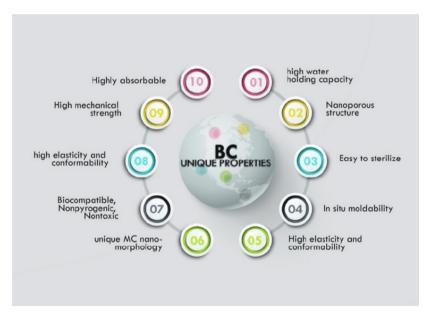
Figure 1. Schematic classification of biopolymer types [3]

Unlike plant cellulose, which is generally mixed with lignin, hemicellulose, and pectin, bacterial cellulose is extremely pure [4]. In terms of the degree of polymerization, bacterial cellulose typically has a degree of polymerization ranging from 2000 to 6000, though some studies have reported values as high as 16,000 or even 20,000 [5]. On the other hand, the degree of polymerization for plant cellulose varies around 13,000 to 14,000 [6]. Bacterial cellulose also exhibits properties such as easier biodegradability and higher hydrophilicity compared to plant cellulose. Additionally, the water retention capacity of bacterial cellulose can reach up to 100 times its dry weight [7].





Due to its high crystallinity, chemical purity, and many other important properties, bacterial cellulose has attracted significant attention, and its ability to be prepared in a short time is a major advantage [10]. Figure 4 illustrates some properties of bacterial cellulose.



# Figure 4. Properties of bacterial cellulose [11]

As a biopolymer with a hydrogel structure, bacterial cellulose (BC) can be modified with other compounds. Numerous studies have focused on tailoring and improving its chemical and physical properties based on the intended application [12-18].



The ability of bacterial cellulose to retain large amounts of water (up to 100 times its dry weight), along with its excellent flexibility and large surface area, is attributed to its fibrils being approximately 100 times smaller in size than those of plant cellulose [16, 19].

Additionally, bacterial cellulose is much thinner than plant cellulose, and its higher surface-to-volume ratio allows for easier interaction with other components [20].



Figure 5. Bacterial cellulose [21]

# **Production of Bacterial Cellulose**

Since most cellulose is obtained from plants, the decreasing availability of plant material and forests has led to research on obtaining cellulose through biotechnological methods in recent years [17, 22]. Bacterial cellulose (BC) is one such material, produced under suitable conditions as a result of cellulose synthesis by acetic acid bacteria. BC is also found in a highly pure form [7].



Carbon and nitrogen sources are among the main factors affecting bacterial cellulose production [23]. By altering various aspects of the culture medium with these factors, bacterial cellulose with desired properties can be produced [15, 23]. As the importance of BC applications increases, various production techniques and methods are being developed [10]. For instance, one study found that using yeast extract as a nitrogen source and glucose as a carbon source resulted in higher cellulose production [24]. In BC production, the temperature is generally maintained between 28-30°C, with an appropriate pH range of 4-7 [14, 25].

The synthesis duration of BC affects the molecular properties of the polymer. Extending this period to six days increases the degree of BC polymer formation, while extending it to 28 days reduces polymer formation and increases polydispersity [26]. Differences in production technology among manufacturers include varying primary carbon source concentrations, surface tension ratios, and fermentation times. For example, Biofill is prepared in two days, while the thicker Gengiflex requires an eight-day fermentation process [27].

#### **Natural Dyeing**

Natural dyes have long been used for applications such as coloring food substrates, leather, wool, silk, and cotton [28]. Historical records indicate that the Chinese used dyes even before 2600 BCE [29]. Natural dyes are derived from roots, stems, leaves of plants, insect shells, and minerals, encompassing all sources naturally obtainable [30].

Although the traditional art of natural dyeing has endured over time, it has experienced a sharp decline due to the affordability and wide availability of synthetic dyes [31]. However, the harmful effects of intermediates and chemicals used in synthetic dye reactions on human health and the environment have rekindled interest in eco-friendly natural dyes [32,33].

Natural dyes are biodegradable and generally less toxic and allergenic than synthetic dyes, making them more environmentally friendly [34]. The synthesis of synthetic dyes depends on petrochemical sources, and some synthetic dyes contain toxic/carcinogenic amines that are not eco-friendly [35,36]. Additionally, unwanted by-products frequently arise during synthetic chemical reactions [37,38]. Certain synthetic dyes release carcinogenic amines when they come into

contact with human sweat, potentially causing various health issues [39-41].

The textile industry, which has a substantial market, requires an average of 700,000 tons of dyes for product coloring, leading to significant amounts of unused synthetic dyes being discharged into rivers, lakes, and groundwater [42].

Therefore, using natural dyes for coloring processes represents an excellent opportunity for value-added production due to their environmental compatibility [43]. Although the natural dye market is relatively small, it is showing an expansion trend [44]. Currently, the global market for plant-based dyes is valued at \$1 billion, with an extraordinary annual growth rate of 12% [45].

## Sources and Applications of Natural Dyes

Common natural dyes include chlorophylls, carotenoids, flavonoids, coumarins, indigoids, and others [46]. Natural dyes are pigments derived from mineral, animal, and plant sources [47]. Plants contain phenolic compounds (phenolic acids and flavonoids), with many flavonoids known to impart color to food. Natural dyes can be extracted from various parts of plants, such as leaves, fruits, seeds, flowers, bark, and roots [48].

Plant-based dyeing involves three main steps:

- Extracting the dye from the plant material.
- Establishing a bridge between the dye and the fabric using a mordant.
- The actual dyeing process [49].

Most natural dyes require mordants in the form of metallic salts to create affinity between the fiber and the pigment [50]. Mordanting, the process of forming a bond between the dye and the fiber, is a pre-dyeing step that makes the fiber more dyeable [51]. Commonly used metal salts include alum, potassium dichromate, chromium, tin chloride, copper sulfate, zinc sulfate, tannin, and iron sulfate [52]. Significant research has been conducted to develop non-toxic and eco-friendly mordants [53]. Mordants enhance fabric uptake, improve color, and increase lightfastness [54].



#### **Citrus Peel as a Natural Dye Source**

Citrus peel consists of two layers: the flavedo and albedo. The flavedo is the outermost thin layer, with colors ranging from yellow to orange-red, and contains carotenoid pigments and cells that produce essential oils [55].

Citrus peels contain two types of natural pigments with different polarities: oil-soluble carotenoids and water-soluble yellow pigments. These pigments are valuable natural colorant sources that could replace synthetic dyes and provide additional coloring to food products. Lemon flavedo contains carotenoids such as xanthophylls (oxygen-containing carotenoids) and carotenes (oxygen-free carotenoids). Xanthophylls are compounds responsible for the typical yellow color of lemon peels [56].

Lemon peel by-products also contain flavonoids and related metabolites, such as naringin, hesperidin, narirutin, and neoeriocitrin. Eriocitrin, a flavanone found in flavedo ( $1002 \pm 65.2 \text{ mg/kg}$  dry weight), is primarily responsible for the coloring of extracts, alongside some carotenoids [57].

The main color-producing compound in orange peel is  $\beta$ -carotene (C40H56), a yellow-red pigment with forty carbon atoms and multiple conjugated double bonds, making it a natural dye source [58].

#### MATERIAL AND METHODS

#### Material

In this study, 100% cotton fabric with enhanced hydrophilicity and bacterial cellulose were used. Citrus peels, specifically lemon and orange peels, were obtained from companies that process citrus fruits, such as juice producers, and separate the peels as waste. Potassium Aluminum Sulfate (KAl  $(SO_4)_2 \cdot 12H_2O$ ) mordant was used in this study.

#### METHOD

#### **Extraction from Dyestuff**

The collected citrus peels were dried at 60°C for 36-48 hours, then ground into smaller pieces using a grinder. To obtain the extract, the prepared citrus peels were weighed separately and soaked in distilled water at 18-22°C for 24 hours. After 24 hours, they were heated on a hot plate to reach boiling temperature within 10 minutes. The peels were then boiled in distilled water at 100°C in a beaker on the hot plate for 60 minutes, with the amount of water lost to evaporation monitored and replenished every 15 minutes.

After boiling, the resulting extract was left to rest in this bath for 24 hours before being filtered. During the dyeing process, the amount of extract corresponding to 5 g of plant material per 1 g of material was calculated proportionally, using 500% of the material weight in dye.

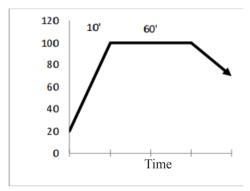


Figure 6. Preparation of dyeing extract

#### **Natural Dyeing Method**

Plant-based cellulose (100% cotton fabric) and bacterial cellulose were dyed using Potassium Aluminum Sulfate (KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O) mordant at a 10% concentration and Sodium Chloride (NaCl) at a 6% concentration, based on the material weight. Color differences were compared separately for mordant-free



dyeing and between plant-based and bacterial cellulose, enabling a comparative analysis of the effects of PAS mordant on the dyeing of plant-based and bacterial cellulose.

The dyeing process was conducted using a conventional method:

- 500% extract
- 100°C
- 60 minutes
- 10% Potassium Aluminum Sulfate (PAS)
- 6% Sodium Chloride (NaCl)
- 1:100 liquor ratio
- 3-hour resting, rinsing, and drying

# Vitamin C Application

Half of the dyed fabrics were treated with vitamin C, aiming to improve lightfastness. The cellulose-based materials, with and without vitamin C treatment, were compared separately to evaluate the effect of vitamin C on lightfastness in bacterial cellulose and plant-based cellulose.

Vitamin C Application Content:

- 1g/L Vitamin C
- 70 Degrees
- 30 min
- 1/100 Flotte
- Rinse and dry

# **Fastness Tests Applied to Fabrics**

# Washing Fastness

The wash fastness of the test samples was evaluated in accordance with the ISO 105-C06 standard using a wash fastness testing machine (Gyrowash Washer Tester). The assessment was conducted using a reflectance spectrophotometer according to the ISO A05 standard. ECE phosphate-free standard detergent was used in the wash fastness tests. The evaluation was carried out using the gray scale and staining scale.

#### Wet-Dry Rubbing Fastness

The rubbing fastness was tested according to the ISO 105-X12 standard using a rubbing fastness testing device (Test T600SH Manual Rubbing Fastness Tester, crockmeter). The evaluation was conducted using the staining scale, and a standard cotton fabric (compliant with TS 717 EN ISO 105-X12) was used as the rubbing cloth.

#### **Light Fastness**

The dyed cellulose-based samples were subjected to lightfastness testing according to the ISO-105-B02 standard using a Xenon lamp in the Solarbox 1500E aging chamber.

## **Color Measurement**

The color measurements of the dyed cellulose-based samples were conducted using a Konica Minolta CM-700d spectrophotometer according to the CIELab system. The color measurements were performed with a 10° standard observer and D65 light source.

## (L\*, a\*, b\*) Color Values Obtained as a Result of the Study

## $L^* = Lightness$

 $a^* = \text{Red} / \text{Green coordinate} + a^* \text{ red}, -a^* \text{ green}.$ 

b\*= Yellow / Blue coordinate +b\* yellow -b\* blue.

K and S = Express the absorption and scattering coefficients of the film layer.

SCI = Indicates measurement including total reflection.

GLOSS= Indicates the degree of brightness.

$$K/S = \frac{1 - SCI^2}{2 * (\frac{SCI}{100})}$$



## FINDINGS

The color differences were comparatively examined in terms of mordantfree dyeing, dyeing with the addition of vitamin C, and dyeing on plant-based cellulose and bacterial cellulose. The effects of PAS mordant and vitamin C on the dyeing of plant-based cellulose and bacterial cellulose were analyzed in a comparative manner.

Table 1. Dyeing of cotton material with temoti peer and orange peer									
Dyeing cotton material with lemon peel	L*	a*	b*	SCI	Gloss	K/S			
Conventional dyeing with mordant	89.56	-1.59	12.9	83.6	38.61	0.016086124			
Conventional dyeing without mordant	89.56	-0.72	12.2	84.71	38.81	0.013799085			
Conventional dyeing with vitamin C mordant	89.56	-0.57	8.99	84.62	39.39	0.013976861			
Vitamin C. Conventional dyeing without mordant	89.56	-0.31	8.89	84.03	39.88	0.015175586			
Dyeing cotton material with orange peel	L*	a*	b*	SCI	Gloss	K/S			
Conventional dyeing with mordant	87.74	-1.82	18.11	82.28	35.88	0.019081089			
Conventional dyeing without mordant	89.33	-0.71	11.73	83.85	39.4	0.015552922			
Conventional dyeing with vitamin C mordant	90.03	-0.89	12.25	84.9	37.98	0.013428151			
Vitamin C. Conventional dyeing without mordant	90.45	-0.25	8.71	85.03	39.93	0.013177755			

When Tables 1 and 2 are examined, it can be observed that the color yield of bacterial cellulose is better than that of cotton fabric. This indicates that bacterial cellulose has a superior dye uptake. The color yield values of bacterial cellulose



dyed with orange peel showed better results compared to those dyed with lemon peel. The addition of vitamin C positively influenced the color yield in bacterial cellulose dyeing. Materials dyed without mordant were found to have lower color yield compared to those dyed with mordant.

Staining bacterial cellulose material with lemon peel	L*	a*	b*	SCI	Gloss	K/S
Conventional dyeing with mordant	77.69	1.52	17.35	67.34	30.22	0.079200743
Conventional dyeing without mordant	78.26	1.19	14.7	65.76	29.35	0.089140633
Conventional dyeing with vitamin C mordant	75.93	2.66	16.53	65.5	25.74	0.090858779
Vitamin C. Conventional dyeing without mordant	78.42	1.28	13.28	65.24	31.8	0.092600981
Staining bacterial cellulose material with orange peel	L*	a*	b*	SCI	Gloss	K/S
Conventional dyeing with mordant	77.63	1.05	21.03	67.37	27.31	0.079020105
Conventional dyeing without mordant	76.76	1.41	19.55	66.03	27.89	0.087381561
Conventional dyeing with vitamin C mordant	72.17	3.55	17.77	61.22	22.32	0.12282656
Vitamin C. Conventional dyeing without mordant	72.69	3.22	14.62	59.19	22.61	0.140687287

Table 2. Staining of bacterial cellulose material with lemon peel and orange peel

Regarding gloss values, bacterial cellulose exhibited lower gloss compared to cotton. Among bacterial cellulose materials, those dyed with lemon peel had higher gloss values, indicating greater brightness, compared to those dyed with orange peel.



The comparative light and rub fastness values of cotton and bacterial cellulose materials dyed with lemon peel and orange peel are presented in Table 3. Due to the use of very thin bacterial cellulose in this study, the rub fastness test could only be performed on cotton fabrics.

When Table 3 is examined, it is observed that dyeing methods using lemon peel provided better light fastness results compared to those using orange peel. In lemon peel dyeing, the light fastness values of bacterial cellulose were found to be better than those of cotton. Additionally, the application of vitamin C in lemon peel dyeing yielded better fastness results compared to materials without vitamin C treatment.

Dyeing with lemon peel	Light	Fastness	Rubb Fastn	Ŭ I
	Cotton	Bacterial Cellulose	Cott	
		Centulose	Wet	Dry
Conventional dyeing with mordant	3	4	5	5
Conventional dyeing without mordant	2-3	4	5	5
Conventional dyeing with vitamin C mordant	3-4	4	5	5
Vitamin C. Conventional dyeing without mordant	3-4	4	5	5
Dyeing with orange peel	Cotton	Bacterial	Cott	on
		Cellulose	Wet	Dry
Conventional dyeing with mordant	2-3	4	5	5
Conventional dyeing without mordant	4	3	4	5
Conventional dyeing with vitamin C mordant	3-4	4	4	5
Vitamin C. Conventional dyeing without mordant	2-3	4	4	4

 Table 3. Light fastness values of dyeing with lemon peel and orange peel



When Table 4 is examined, it is observed that dyeing methods using lemon peel provided better light fastness results compared to those using orange peel. In lemon peel dyeing, the light fastness values of bacterial cellulose were found to be better than those of cotton. Additionally, the application of vitamin C in lemon peel dyeing yielded better fastness results compared to materials without vitamin C treatment.

Similarly, in orange peel dyeing methods, the light fastness values of bacterial cellulose were observed to be superior to those of cotton. It was also determined that the application of vitamin C produced better results in lemon peel treatments compared to orange peel treatments.

Cotton material dyeing	Washing fastness								
methods (lemon)	Color Change	Staining Fastness							
Conventional dyeing with mordant	4	WO	PAN	PES	PA	СО	СА		
Conventional dyeing without mordant	5	5	5	5	5	5	5		
Conventional dyeing with vitamin C mordant	5	5	5	5	5	5	5		
Vitamin C. Conventional dyeing without mordant	4	5	5	5	5	5	5		
Cotton material dyeing	Color Staining Fastness								
methods (orange)	Change	WO	PAN	PES	PA	<i>C0</i>	CA		
Conventional dyeing with mordant	5	5	5	5	5	5	5		
Conventional dyeing without mordant	4	5	5	5	5	5	5		
Conventional dyeing with vitamin C mordant	5	5	5	5	5	5	5		
Vitamin C. Conventional dyeing without mordant	4	5	5	5	5	5	5		

Table 4. Dyeing of cotton material with lemon peel and orange peel



Regarding rub fastness test results, materials dyed with both lemon peel and orange peel showed similar fastness results. Generally, dry rub fastness values were higher than wet rub fastness values. Comparing lemon peel and orange peel, dyeing with lemon peel provided better rub fastness results than dyeing with orange peel. The washing fastness results of bacterial cellulose and cotton dyed with lemon peel and orange peel are given in Table 4 and Table 5.

Bacterial cellulose material	Washing fastness								
dyeing (lemon)									
	Color	Staining Fastness							
	Change	WO	PAN	PES	PA	CO	CA		
Conventional dyeing with mordant	4	5	5	5	5	5	5		
Conventional dyeing without mordant	5	5	5	5	5	5	5		
Conventional dyeing with vitamin C mordant	5	5	5	5	5	5	5		
Vitamin C. Conventional dyeing without mordant	4	5	5	5	5	5	5		
Bacterial cellulose material	Color	Staining Fastness							
staining (orange)	Change	WO	PAN	PES	PA	CO	CA		
Conventional dyeing with mordant	4	5	5	5	5	5	5		
Conventional dyeing without mordant	5	5	5	5	5	5	5		
Conventional dyeing with vitamin C mordant	5	5	5	5	5	5	5		
Vitamin C. Conventional dyeing without mordant	4	5	5	5	5	5	5		

Table 5. Bacterial cellulose material dyeing with lemon and orange peel

Excellent results were obtained in the wash fastness of bacterial cellulose and cotton, achieving the best fastness values across six different fabric types on the scale of 1 to 5. Bacterial cellulose provided fastness results that were at least as

good as those of cotton. Similar results were generally observed in the dyeing of bacterial cellulose with both lemon peel and orange peel. The effect of the mordant was also similar to that observed in non-mordanted materials.

# CONCLUSION

Bacterial cellulose, synthesized by certain microorganisms, is a multifunctional nano-biomaterial composed of linear glucan molecules bound by hydrogen bonds and appears similar to plant cellulose. Unlike plant cellulose, which is generally mixed with lignin, hemicellulose, and pectin, bacterial cellulose is extremely pure. The most important feature of the natural dyeing method is that it minimizes water and energy consumption during the dyeing process, thereby significantly reducing the amount of wastewater generated.

Both plant cellulose (100% cotton fabric) and bacterial cellulose were dyed using Potassium Aluminum Sulfate (KAl(SO4)2·12H2O) as a mordant at a concentration of 10% and Sodium Chloride (NaCl) salt at a concentration of 6%, based on the weight of the material. The color differences were compared both in dyeing without mordant and between plant cellulose and bacterial cellulose, allowing for a comparative analysis of the effects of PAS mordant on the dyeing of plant and bacterial cellulose. Conventional dyeing is a traditional method performed at high temperatures for a certain duration. Half of the dyed fabrics were treated with Vitamin C at 70°C for 30 minutes using 1 gram of Vitamin C per liter at a 1:100 liquor ratio. The application of Vitamin C aimed to improve the light fastness of cotton fabrics. Test results showed that the color yield of bacterial cellulose was much better than that of cotton fabric, demonstrating that bacterial cellulose has a better color uptake. The addition of Vitamin C during the dyeing of bacterial cellulose had a noticeable effect on color yield, positively enhancing it. The light fastness test showed that materials without mordant provided better fastness results. Based on the tests conducted in this study, it is observed that bacterial cellulose could be a good alternative to plant cellulose.

Considering all the results obtained in the study, bacterial cellulose demonstrated better values in terms of color yield and other fastness tests compared to cotton fabric. These findings indicate that bacterial cellulose could be a superior alternative to cellulose in similar applications.



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