Chapter 1

Introduction

1.1 Background

Humans have used dyes since prehistoric times, eventually developing them for various industries, such as textiles, cosmetics, pharmaceuticals, photography, plastics, and others. To enhance the color intensity and stability of those materials, people began to invent synthetics for larger-scale industries (Ardila-Leal et al., 2021). However, synthetic dyes are major sources of aquatic pollutants related to their potential to contaminate the water bodies and the organisms through the effluent discharge from factories (Slama et al., 2021). In most cases, the waste from the common artificial dyes—azo, indigo, xanthene, anthraquinone—is noticeably harmful to the environment due to their inhibitive shading for aquatic photosynthesis, toxicity risk, and carcinogenicity (Garg & Chopra, 2022).

Surprisingly, the aforementioned dyes become more hazardous with additional effects, including iridescence to add visual value. In this case, iridescence is a shimmer effect resembling the visual effect of pearls that can demonstrate multiple colors depending on viewing and illumination angles. It is generally difficult to imitate with sustainable materials, as the current manufacturing source is mainly from mica (aluminum silicate or potassium aluminum silicate), further bringing toxic traces of iron, lithium, and magnesium (Uddin, 2021) that often end up in the environment. Workers also face severe hazards from the poisonous residues and dust, underscoring the need to replace synthetic dyes with a more eco-friendly alternative (Pol, 2023). If these issues are not addressed, not only will they harm those directly affected, but they will also contribute further to the existing prediction that 47% of the world's population will endure clean water scarcity by 2030 (Al-Tohamy et al., 2022).

For the past decades, research has focused on the solution through a bio-based dyes approach using natural extracts, such as turmeric (yellow), algae *Spirulina platensis* (green), fungi *Ganoderma* *lucidum* (red), and many more. However, these natural options are limited to solid colors (Budipramana et al., 2023; Ciptandi et al., 2021; Samantha & Singhee, 2023). Natural structural colors can produce optically unique colors from the interaction between a certain microscopic structure and light. A good example of the material is guanine crystals, organic biogenic crystals with an outstanding refractive index in various organisms. This can include the color-changing skin of spiders, fish, and amphibians, and the scallop eyes. However, the sustainability is still limited by their role in the ecosystem and reproduction rate (Wagner et al., 2023).

A similar kind of material was also reported by Pavan et al. (2022), who revealed that prokaryotes also potentially produce guanine crystals. However, studies on prokaryotes are minimal compared to eukaryotes. Pavan and co-workers showed that several bacterial species: *Aeromonas salmonicida* (subsp. *masoucida, salmonicida, smithia, achromogenes, pectinolytica*)*, A. aquatica, A. eucrenophila, A. tecta, A. encheleia, A. caviae, A. sanarellii, A. taiwanensis, A. rivipollensis, A. media, A. bivalvium, A. rivuli, A. molluscorum,* and *A. simiae* could produce guanine crystals, proving the feasibility. Considering their excellent reproduction rate through binary fission, more straightforward nutritional needs, and a minimum space requirement, bacterial guanine crystals are a promising alternative (Weissman et al., 2022). Thus, they are likely to substitute synthetic dyes and iridescent sources with a more bio-based and sustainable approach, providing the special optical properties that allow industries to still achieve the desired visual effect.

Unlike Pavan et al. (2022), who focused more on the identification of guanine crystal-producing species, this study shifted the aim to learn more about the optimization through a nutritional approach using an *in silico* tool, KBase (Arkin et al., 2018), for guanine accumulation. Moreover, nitrogen, a primary component in guanine structure, as suggested by Hu et al. (2023), was adjusted during the metabolic simulation in this study. Additionally, a light simulation using NanoHUB-PhotonicsRT (Ishii et al., 2014) was employed to characterize the material based on its reflectance and transmittance. The findings may act as a preliminary study for a future wet-lab research that can better validate the *in silico* output. It may effectively contribute to cost and time reduction in future wet lab experiments (Yusuf, 2023). However, one should also acknowledge that this *in silico* study was employed as a prediction that simulated the factual basis to disclose the limitations in the target metabolism. Despite the dissimilarity with Pavan et al. (2022), the study still involved *Aeromonas salmonicida* subsp. *pectinolytica* and *Shewanella oneidensis* MR-1, which had been proven to produce guanine crystals, and *Escherichia coli* BW25113, which did not (negative control)—representing the past discoveries with complete genetic information to work with.

By the end of the study, the collected data focused on demonstrating the impact of nitrogen concentrations and guanine accumulation, while also considering the effect of the predicted accumulation flux and the optical response. The final output determined whether the crystals can be helpful as they are or must be further modified to generate high-quality structural coloration.

1.2 Objective

The research intends to solve several points, as follows:

- To predict the effect of different nitrogen concentrations in *Aeromonas salmonicida* subsp. *pectinolytica* and *Shewanella oneidensis* MR-1 for guanine crystal synthesis through the flux balance analysis (FBA) modeling.
- To characterize the optical properties of the simulated guanine accumulation to light.

1.3 Hypothesis

For the first objective, the null hypothesis (H_0) was defined as different nitrogen concentrations do not significantly affect guanine accumulation, whereas the alternative one (H_1) suggested that they do. Furthermore, the second objective was hypothesized not to show any correlation between the guanine accumulation and reflection-refraction values (H_0); alternatively, there might be a relationship between the two (H_1).