fermentation

Editorial

Pigment Production Using Submerged Fermentation

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There has been a continuous increase in consumer awareness regarding the availability of natural, sustainable, biodegradable options in all sectors, including food, cosmetics, pharmaceuticals, textiles, painting, printing inks, etc. The growing use of natural pigments in various industries is expected to drive the market growth rate and boost its demand globally [1,2]. To meet these global demands, microorganisms have been considered and are widely being researched as a promising niche for pigment production, owing to their vast diversity in nature, and easy, large-scale production. Microbial oddities are attracting more attention to research, not only due to their wide potential as pigments but also their diverse chemical structures possessing bioactive properties, such as antimicrobial, antitumor, and anticancer properties [3]. This indicates that future generations will depend on microbial pigments more than synthetic colorants for a sustainable livelihood.

Microbial pigments are secondary metabolites produced using two main techniques, solid-state fermentation (SSF) and submerged-state fermentation (SmF), yet both methods are influenced by different physiochemical parameters. Among the two methods, submerged fermentation is the more widely employed practice in industrial fermentation considering its easiness with regard to substrate utilization, control of process parameters, recovery of targeted metabolites, etc. [4]. On those grounds, the journal *Fermentation* opened this Special Issue intended to cover recent interesting findings on new potential pigment-producing microbial sources, low-cost substrate utilization as nutrient sources, possible scale-up studies along with process optimization, and efficient technologies for high pigment recovery. Those are the key areas under focus on economic pigment production which paves the way for industries to mass-produce fermentation-derived microbial natural colors. This Special Issue collected a total of 13 articles, which includes 1 review and 12 research articles.

To start with, the review by Ramesh et al. [5] explores the importance of extensively studied pigmented microbes for their use in industry as colorants, in human health with bioactivities, and in the environment by mitigating climate change. The article highlights the spread of microbial sources at various geographic locations and emphasizes the link to carry out evolution studies. It also focused on the implications of submerged fermentation for pigment production, and to showcase that, a table with substrate concentration and media composition to induce pigment yield for various microbes was presented. However, the authors pointed out the need to perform biosynthetic pathway studies to understand the genes and enzymes responsible for pigment production. The need was also prioritized to conduct more studies to prove its biological properties associated with pigments.

In line with circular bioeconomy and economic sustainability, one of the main interesting areas focused on nowadays is the use of agro-industrial wastes as low-cost substrates for carrying out microbial fermentation to produce valuable secondary metabolites. With this interest, Pyter et al. [6] studied carotenoid production in *Paracoccus* strains using wheat straw and pinewood dust which are two abundant lignocellulosic wastes. The authors employed an alkali pretreatment approach followed by enzymatic saccharification which resulted in the highest carotenoid production. Similarly, Tran et al. [7] carried out astaxanthin production in *R. toruloides* G17 using waste molasses, and it was found to exhibit potential

Citation: Venkatachalam, M. Pigment Production Using Submerged Fermentation. *Fermentation* **2024**, *10*, 91. https://doi.org/10.3390/fermentation10020091

Received: 23 January 2024
Accepted: 1 February 2024
Published: 4 February 2024

antioxidant and anticancer activity against three cell lines—HeLa, A549, and MCF7—along with highest astaxanthin yield. Maia et al. [8] aimed to investigate carotenoid production in *R. glutinis* using manipueira as a low-cost substrate. Their findings from HPLC-DAD analysis revealed seven major carotenoids produced, while lutein and 5,8-epoxy-lutein were identified for the first time in the biomass of *R. glutinis*. All the above-mentioned research articles strengthened the dual benefits of using feedstocks in terms of low production cost along with managing the waste stream. Additionally, it showcased the production of target compounds with high yield.

Over the past decade, immobilized cell fermentation has been viewed as a promising biotechnological tool and provides an efficient approach in terms of productivity, stability, extraction, and process control. Ruiz-Sánchez et al. [9] worked with immobilized cells of *T. atroroseus* GH2 using different support material to produce red pigments. The authors reported that at improved conditions, pigment production was 30% higher than using free cells, accompanied by a long-lasting immobilization activity (99.01 $\pm$ 0.37%). This study also evaluated the process kinetics and demonstrated that the production could continue for three batches by recycling the immobilized biomass. Likewise, Shi et al. [10] attempted to combine extractive fermentation in nonionic surfactant (Trition X-100) and immobilized cell fermentation for the continuous production of *Monascus* yellow pigments, as well as studying its effect on cell growth and pigment stability. As a result, it was revealed that the immobilized cells could be reused for at least seven batches, and this strategy provides a novel strategy for the continuous and easy extracellular extraction of pigments.

To make any process industrially viable, it is necessary to understand the process conditions and challenges encountered in higher production volumes by performing scale-up studies. Venkatachalam et al. [11] conducted scale-up studies in a 5L stirred tank bioreactor for a marine-derived fungi, *Talaromyces albobiverticillius* 30548, which yields red azaphilone pigments. The rate-limiting factors while performing at such a scale were listed, and it was discussed how those factors affected the overall performance of the fermentation process including biomass and pigment yield. In the same way, Bregmann et al. [12] used a wood-decaying basidiomycete, *Laetiporus sulphureus*, to study and compare the production yield of yellow-orange pigments in a 7L bioreactor and noted a 19% increase in shake flask cultivation. Another study performed by Bregmann et al. [13] was devoted to producing hispidin for dye textiles using *Inonotus hispidus* by running a comparative, parallel fermentation in a stirred tank and wave bag reactor. A combination of illumination, chemical stressor, precursor supplementation, and fungal cell morphology was taken altogether as factors, and its influence on fermentation yield was discussed in detail. Further, with the obtained yellow hispidin, silk and wool materials were dyed and tested for light stability, and a noticeable color change was reported. Collectively, this work paved the way to further improve its use in other applications, and the authors suggest examining the potential bioactivities associated with it.

Another area of interest includes bioremediation in which the microbes secrete enzymes to breakdown harmful contaminants. Pham et al. [14] isolated and characterized an enzyme (lipid peroxidase)-producing soil bacteria (*Bacillus* sp. React 3) that efficiently decolorizes methylene blue dye. The decolorization efficiency was determined by conducting enzyme activity and decolorization assays. Moreover, it was dependent on several factors such as temperature, pH, inoculum size, carbon and nitrogen sources, and initial dye concentration since all these parameters contribute to creating favorable conditions for microbial activity and the successful degradation of colorants. In a similar manner, two field isolates of the green microalga *Chlamydomonas reinhardtii* were chosen by Intha et al. [15] to exploit its tolerance to cell growth and for pigment production in the presence of environmental stressors, namely Norflurazon and ZnO nanoparticles. Examining two different strains, reactions to both environmental stressors were distinct and exhibited different behavioral responses, and pigment yield was well demonstrated in this work.

Microalgae can produce pigmented bioactive compounds using photosynthesis which is unmatched by synthetic pigments. In a research paper, Lee et al. [16] highlighted the application of *Pseudoalteromonas haloplanktis* to ferment three valuable algae (*Colaconema formosanum*, *Sarcodia suae*, and *Nostoc commune*) to obtain pigments and polysaccharides. Two-stage fermentation was executed to investigate the production of compounds and extraction efficiency depending on the individual algal fermentation potential. Phycophiliproteins were collected efficiently using one-step fermentation (6 h), whereas fermentation with fragmentation at the second stage brought three times higher polysaccharides, resulting in a higher degree of extraction.

Many literature studies report that microorganisms not only produce a palette of colors but also exhibit a wide range of diverse biological activities. One research paper deals with the biological activities of endophytic fungi, *Monascus purpureus* CPEF02, and its mechanism of action against anthropogenic pathogens. In this work by Kaur et al. [17], pigmented methanol extract exhibited antioxidant activities (14.42 µg/mL IC_{50}), and pigmented ethyl acetate extract showed antimicrobial activity against both bacterial and fungal pathogens. For compounds such as Monasfluore B, monascin, purpureosone, and rubropunctamine of the methanolic extract reported in this study are consistent with the reported activities, which is supported by existing literature studies but others need more experimentation.

This editorial is a summary of all the collected articles in this Special Issue, which covers the potential of different microalgae, bacteria, and fungi to produce industrially important compounds using submerged fermentation.

I am grateful to all the participating researchers, authors, and professors who have contributed to the success of this Special Issue. I would also like to take this opportunity to thank all the reviewers, academic editors, Editors-in-Chief, and the whole MDPI team involved in editing, managing production, and the website.

Conflicts of Interest: The author declares no conflicts of interest.

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