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Advancements in biofeedback photobioreactors: using the language of light deciphered from the organisms themselves

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Abstract

This paper presents an innovative exploration of biofeedback photobioreactors, which utilise the "language of light" decoded from photosynthetic organisms themselves. Addressing the inherent inefficiencies in photosynthetic conversion under various environmental conditions, the study delves into the potential of optimising abiotic factors in such systems. The core principle involves chlorophyll *a* fluorescence as a real-time indicator of photosynthetic activity, offering a non-invasive, comprehensive communication method between the researcher and the microorganism. By integrating this approach with advanced machine learning techniques, the paper proposes a method for deconvoluting complex fluorescence signals unique to each species. This approach not only holds the promise of enhancing the efficiency of photosynthetic microorganisms in controlled environments like bioreactors but also paves the way for significant advancements in sustainable biofuel production and other biotechnological applications. The paper underscores the importance of interdisciplinary research in overcoming the challenges of photosynthetic efficiency and highlights the potential of biofeedback photobioreactors to revolutionise the field of algal biotechnology.

Introduction

Photosynthesis, the fundamental process by which plants, algae, and some bacteria convert light energy into chemical energy, has immense implications for both natural ecosystems and human industries. Despite its critical role, the efficiency of photosynthetic conversion, particularly under varying environmental conditions, remains suboptimal (Falkowski et al., 2017; Lin et al., 2016; Melis, 2009). On a global scale, phytoplankton convert approximately 35% of absorbed photons to chemical energy through photosynthesis, while the majority, about 60%, are dissipated as heat, indicating a relatively low efficiency of photosynthetic conversion (Lin et al., 2016). Furthermore, under ideal conditions, the maximum efficiency of plant photosynthesis in sunlight is 13%, however, when accounting for real-world factors such as measured quantum yields and absorption factors, the efficiency estimate drops to 9.3%, highlighting the intrinsic limitations and suboptimal efficiency of photosynthesis in natural conditions (Bolton and Hall, 1991). This inefficiency works well in natural environments but poses a significant challenge in controlled environments, such as bioreactors used in biotechnological applications.

The advent of biofeedback photobioreactors presents a promising solution to optimise biotic and abiotic factors that impact photosynthetic microorganisms. Optimisation of light utilisation via biofeedback has been demonstrated in higher plants (Ahlman et al., 2017; Van Iersel et al., 2016) but to date this research has not been translated in the microalgal research community. By integrating a feedback loop

between the photosynthetic organism and the light source, these systems promise a more efficient energy conversion process. The adoption of such technology could herald a significant leap forward in enhancing energy use efficiency and yield, with broad implications for sustainable production of biofuel (Brennan and Owende, 2010; Hoang et al., 2023; Peng et al., 2020; Rafa et al., 2021), pharmaceuticals (Lu et al., 2024), human consumption (Yang et al., 2024), nutraceuticals (Nicoletti, 2016), aquaculture feed (Han et al., 2019) and wastewater treatment (Srimongkol et al., 2022). This paper presents an exploration of the biofeedback photobioreactor concept, delving into the technological paths that might lead to such a breakthrough in the study of and applications with photosynthetic microorganisms.

Biofeedback: a two-way conversation

Simply put, the concept of biofeedback is a two-way communication between a microorganism and the researcher. For such a system to work we need to have an effective communication system that allows us to get information from the microorganism (1) in real-time, (2) non-invasively, and (3) the information needs to be complex enough to describe as many internal biological processes as possible.

At the core of this innovative approach is the utilisation of chlorophyll *a* fluorescence as a real-time indicator of the photosynthetic and by extension biological status of the cell. Chlorophyll *a* fluorescence is a key non-invasive indicator of photosynthetic activity, specifically of photosystem II (PSII), and is extensively used in algal and plant research (Papageorgiou and Govindjee, 2004). The principle of chlorophyll *a* fluorescence analysis is based on the fact that light energy absorbed by chlorophyll molecules can have three fates: it can drive photosynthesis (photochemistry), be dissipated as heat, or be re-emitted as light — the latter is chlorophyll *a* fluorescence. These processes are interdependent, meaning an increase in the efficiency of one leads to a decrease in the others. Therefore, by measuring the yield of chlorophyll *a* fluorescence, insights can be gained into changes in the efficiency of photochemistry. The method is responsive enough to provide insight on the impact of environmental variables on the cell, which most commonly include, but are not limited to, light intensity (Herdean et al., 2022), light spectra (Bernát et al., 2021; Herdean et al., 2021), temperature (Herdean et al., 2023; Salleh and McMinn, 2011), pH (Behrendt et al., 2020), and nutrients (Nagi et al., 2023). Furthermore, due to the complex interconnectivity of cellular processes, chlorophyll *a* fluorescence, emitted during photosynthesis, serves as a window into the inner workings the cell that go beyond the chloroplast (Bailleul et al., 2015). Data derived from such measurements will form the communication "language" that the photosynthetic microorganism uses in this two-way conversation.

One may ask at this point: given that the method of communication exists, why are there no biofeedback photobioreactors in use? To appropriately answer this question, there are two challenges with chlorophyll *a* fluorescence that need to be pointed out: first, the signal is complex and arguably not fully understood; it's widely accepted that it provides rich insight into the cell's biology, but we only understand small fractions of that signal. Second, each species has its own unique biological response, which results in a fluorescence signal which, to a large

extent, is unique to that species. While not an easy undertaking, this is actually a solvable problem. It is worth giving some examples of fluorometry data used for optimisation, such as growth temperature (Ranglová et al., 2019), glycogen content (Lakatos et al., 2021), biomass production (Masojídek et al., 2011), and generally as an indicator for nutrient stress (Parkhill et al., 2001). A strategy similar to that used in medical sciences (Sagar et al., 2020) could be applied here. Researchers could utilise supervised machine-learning approaches such as neural networks, random forests, or similar architectures, combined with systematic experimentation. By exposing microorganisms to a wide range of environmental conditions, researchers could record specific fluorescence responses, generating data that could train machine-learning algorithms for more accurate classification. This experimental strategy does not require significant a priori knowledge of the microalgae properties or photo-physiological properties; it requires, however, an additional measurement of cell health which will be used to classify the fluorescence measurements. This approach will provide a "Rosetta stone"-type of deconvolution of the chlorophyll signal, enabling the two-way communication. Partial success has already been shown in using machine-learning to deconvolute the significance of fluorescence data from satellite imagery (Bartold and Kluczek, 2023; Liu et al., 2022) and terrestrial measurements (Rybka et al., 2019). This can be taken a step forward by using a generative adversarial network trained on the experimental data to generate signals that the organisms can likely produce but have not been recorded in the initial dataset (Chen et al., 2022).

Assuming the means of communication are resolved at single-cell level, an additional complication arises: the unavoidable heterogeneity found in a population of cells. Realistically, a biofeedback system will work with asynchronous and heterogeneous cell cultures. This means that at any given time, the population is composed of cells at different stages of development, which will likely make signal deconvolution more difficult. Just as before, this is not an unsolvable problem.

Communication with populations: from 1 to many cells

In advancing the field of biofeedback photobioreactors, a key consideration is the heterogeneity inherent in populations of microalgae. As shown by the elegant use of a microscope fitted with a fluorometer (Trampe et al., 2011), microalgal populations exhibit considerable variation in fluorescence response among individual cells. When scaling-up the measurements, this variability averages out and provides a single signal representing the entire population. More recent research demonstrates the utility of microfluidic photobioreactors in observing and cultivating microalgal cells at the single-cell level — a crucial step in understanding population dynamics (Westerwalbesloh et al., 2019). In this context, the transition from individual-cell analysis to population-level communication presents unique challenges and opportunities. A single cell photobioreactor allows for the controlled cultivation of microalgae, providing a platform where individual cells or small aggregates can be studied in isolation under well-defined conditions. This approach is instrumental in discerning the responses of microalgae at

various developmental stages, and to varying environmental stimuli.

Understanding the nuances of individual cellular responses within a population is critical for developing a comprehensive biofeedback system. The rationale behind it is to clarify how complex signals coming from cells in different biological states will average when measuring the whole population. By integrating the insights gained from such microfluidic photobioreactor studies with machine learning algorithms, researchers can unravel the complex web of intercellular communication and response mechanisms. This integration can lead to the development of sophisticated biofeedback systems that can dynamically adapt to the needs of not just individual cells but entire populations.

The heterogeneity observed in microalgal populations underscores the importance of considering individual cellular states and responses. This knowledge is fundamental for achieving effective two-way communication in biofeedback systems. It enables the prediction and modulation of population behaviour, thereby optimising the overall efficiency of the photobioreactor.

Significance and innovation: redefining biotechnological frontiers

The development of biofeedback photobioreactors when completed will not merely be an incremental advancement but a significant leap in biotechnological innovation. This approach holds the promise of addressing one of the most enduring challenges in photosynthesis research: optimising light energy utilisation in varying environmental conditions in real-time. The significance of this innovation extends beyond academic curiosity, potentially revolutionising industries reliant on photosynthetic organisms, such as biofuel production, pharmaceuticals, and food technology.

Innovatively, this technology leverages the dynamic nature of photosynthesis — a departure from traditional static approaches. The term "dynamic nature" refers specifically to the capacity of biofeedback systems to adaptively respond in real time to variations in photosynthetic activity. This contrasts sharply with "static approaches," which represent conventional methodologies that lack the capability to adjust to immediate changes in cellular responses. The introduction of machine-learning and generative adversarial networks to interpret chlorophyll *a* fluorescence signals represents a novel strategy, enabling a more nuanced understanding and control of the photosynthetic process. This innovation could lead to unprecedented improvements in energy efficiency, reducing operational costs and enhancing sustainability in biotech industries.

Challenges and limitations

The history of biofeedback photobioreactors and optimising photosynthetic efficiency has been marked by several key developments. Initial efforts in this field were primarily focused on understanding the basic principles of light interaction with photosynthetic organisms and the design of photobioreactors for efficient microalgae growth (Ahmad et al., 2021). Over time, researchers have explored various reactor designs and light-management strategies to improve photosynthetic efficiency (Janssen et al., 2003). This has included innovations in reactor configurations and light-distribution methods, reflecting a continuous evolution of the technology. Noteworthy photobioreactor designs include tubular

systems (Molina et al., 2001), flat panel (Slegers et al., 2011), thin-layer cascade (Villaró et al., 2022), revolving algal biofilm (Schaedig et al., 2023), and even hybrid designs that separate the dark and light reactions (Deprá et al., 2019). However, the integration of biofeedback mechanisms to dynamically adjust to the photosynthetic organism's needs, represents a more recent and significant advancement in this area.

The technological challenges in the development of biofeedback photobioreactors for optimising photosynthetic efficiency are multifaceted. They involve complexities in accurately interpreting chlorophyll *a* fluorescence data, which necessitates sophisticated algorithms and sensing technologies. Additionally, designing photobioreactors that can dynamically adjust lighting, temperature, nutrients and other conditions in real time to optimise photosynthesis presents engineering challenges. There are examples with partial success where biofeedback has been experimented with (Ifrim et al., 2013; Melnicki et al., 2013) but without use of machine-learning tools and using a limited number of parameters. The next generation of such systems must be capable of rapidly responding to the changing photosynthetic and biological needs of the organisms, requiring advanced control systems and integration of multiple feedback mechanisms. This necessitates a convergence of biotechnology, sensor technology, and control engineering, each with its own set of technical hurdles.

Potential outcomes and impact: a paradigm shift in photosynthetic efficiency

The proposed biofeedback photobioreactor holds the potential to significantly reduce energy losses in algal photosynthesis, marking a paradigm shift in the industry. This breakthrough approach could lead to unprecedented energy savings and enhanced production predictability in algal biotech. Moreover, it offers a direct method to answer scientific queries about photobiology, using the language of light deciphered from the organisms themselves.

The successful implementation of this technology could revolutionise the field of algal biotechnology, contributing to more sustainable practices. It would set a new standard for photosynthetic efficiency, potentially impacting a wide range of applications, from biofuel production to pharmaceuticals. The project's interdisciplinary nature could also pave the way for novel research methodologies, fostering advances across various scientific disciplines.

Conclusion: forging a new path in photosynthetic research and biotechnology

The development of the proposed technology would represent a significant leap in the fields of photosynthetic research, algal biotechnology, and data analytics. This approach not only promises enhanced efficiency in energy conversion but also sets a new benchmark for sustainable and predictable production systems. It underscores the importance of interdisciplinary collaboration and innovation in overcoming historical challenges in photobiology. As we stand at the cusp of this technological revolution, it is imperative to continue exploring and refining these novel methodologies, potentially ushering in a new era of sustainable and efficient biotechnological solutions.

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