Microalgal Phycocyanin Productivity: Strategies for Phyco-Valorization

[Short Title: Microalgal Phycocyanin Productivity for Phyco-Valorization]

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Abstract

Phyco-valorization is the exploitation of microalgae and microalgal chemicals as valuable products. This paper discusses the optimization of microalgal bioreactor-based systems for C-phycocyanin pigment production. Various aspects contributing to system development and enhancement of phycocyanin productivity are described. A wide range of potential microalgal species have been identified for phycocyanin production; the selection of a species for mass culturing can be determined by desired bioreactor trophic mode and symbiotic relations. Research has demonstrated that species amenability to local lighting and climatic conditions, and to variations in bioreactor substrate concentrations and operational parameters, have significant impact on phycocyanin production. The simultaneous optimization of all factors contributing to system productivity may be efficiently accomplished through process modelling. A summary of established models for microalgal phycocyanin production is presented. A suggested strategy for increasing economic viability

of phycocyanin production systems is their application in integrated resource recovery. Through the incorporation of phycocyanin productivity optimization principles within a phycoremediation process, the valorization of waste resources may be achieved. The simultaneous economic potential and environmentally-forward concept of phyco-valorization through phycocyanin production is a promising application of microalgal biotechnology awaiting further development for industrial implementation.

Keywords: Phycocyanin; Integrated Resource Recovery; Microalgae Bioreactor Optimization.

Contents

Nomenclature

Bi: Biot number

K_p: Half-saturation constant for short-term Fe uptake (M)

N_b: Bulk nitrogen concentration (mg/l)

N_g: Nitrogen concentration within the gel bead (mg/l)

N_s: Interfacial nitrogen concentration (mg/l)

r: Radial coordinate (cm)

t: Cultivation time (days) (Equation (2)); Time coordinate (s) (Equations (3) and (4))

X: Algal biomass concentration (g/L)

X: Variable; X_1 = $NaNO_3$ (g/L); X_2 = $CaCl_2$ (g/L); X_3 = Citric acid stock (ml/L); X_4 = Trace metal mix (ml/L)

X_0: Initial algal biomass concentration (g/L)

X_m: Maximum biomass in logistic equation in unit of (g/L)

Y: Phycocyanin production (mg/ml)

α: Ratio of total volume of beads available for liquid mass transfer to volume of medium solution

β_o, β_i, β_{ii}, β_{ij}: Constant process effect in the total, the linear, the quadratic effect of X_i, and the interaction effect between X_i and X_j

μ: Initial specific growth rate in unit of (1/day)

ρ: Uptake rate (mol/cell/s)

ϕ^2: Thiele modulus

Introduction

In recent times the microalgal production industry has capitalized on advances in biotechnology to expand and diversify.[1–8] It now exploits a wider range of species amenable to mass culturing, and their corresponding applications, which go beyond biofuels. For instance, mass cultured microalgae has been valorized as health food products.[9,10] This shift has sparked interest in the development of economical techniques to extract high-value chemical compounds from microalgae. One such class of valuable compound are photosynthetic pigments.[11–17] This article provides insights into the knowledge and the strategies involved with phyco-valorization, the exploitation of microalgae and microalgal chemicals as valuable products, by taking the pigment phycocyanin as an exemplar.

Phycocyanin is a light-harvesting pigment and nitrogen-storing protein found in the prokaryotic cyanobacteria species, as well as in eukaryotic chlorophyta, rhodophyta, and bacillariophyta species.[18] Phycocyanin is a type of chromoprotein (or biliprotein) that absorbs radiation in regions of the visible spectrum where chlorophyll *a* has low absorptivities.[19] According to the naming convention of MacColl[19], four varieties of phycocyanin have been identified, each having a different type or combination of phycobilin (light-capturing pigments, shown in brackets): C-phycocyanin (phycocyanobilin); phycoerythrocyanin or CV-

phycocyanin (phycocyanobilin, phycoviolobilin); R-phycocyanin II or CE-phycocyanin (phycocyanobilin, phycoerythrobilin); phycocyanin WH8501 or CU-phycocyanin (phycocyanobilin, phycourobilin). C-phycocyanin has been identified as a promising candidate for phyco-valorization due to its array of functionalities, which enable many different applications.[20,21] All further references to phycocyanin in this paper relate to C-phycocyanin.

A primary consideration for a potential phycocyanin producer is the selection of a microalgal species amenable to the conditions of the prospective production system. An individual species will demonstrate optimal growth within a specific range of process parameters. Throughout this article, the optimization of such parameters will be analyzed – demonstrating the dynamic range of optimal conditions reported for a number of microalgae species.

Another important consideration is the cost of the microalgal cultivation and harvesting process. Based on sustainability precepts, a potential route to reducing costs is by utilization of waste resources or unused infrastructure. Potential resources that can be drawn include substrate sources, pH adjusting materials, heat sinks, and available light. Phycoremediation is a microalgal technology that has had promising amenability to resource recovery from a variety of waste streams.[22–31] An integrated resource recovery concept may also be beneficial to microalgal phycocyanin producing systems, and will be discussed in this paper.

Applications of Phycocyanin

A critical aspect of phycocyanin that has contributed to its rising popularity for commercial production is its inherent nutraceutical function. One microalga championing the health benefits of phycocyanin is *Arthrospira platensis* (formerly known as *Spirulina*

platensis[32]). This species of cyanobacteria has recently drawn great interest from the health food industry and academia; the integration of *A. platensis* into a wide variety of food products, taking advantage of phycocyanin's nutraceutical attributes, has been explored.[20,21] Table 1 summarizes literature identifying various nutraceutical functions of phycocyanin.

The intense cyan coloration of phycocyanin has led to its use as a colorant for food and cosmetic applications. It is of particular importance to these industries in view of increased consumer preference for natural colorants in place of their potentially toxic synthetic counterparts.[57] Health conscious consumers also benefit from the imparted nutraceutical properties. This trend corresponds with the increased popularity of phycocyanin colorant following its introduction in Japan by Dainippon Ink & Chemicals Incorporated in 1980.[34] This is further exemplified by the recent ruling from the U.S. Food and Drug Administration that allowed the usage of phycocyanin derived from *Spirulina* (former nomenclature) to be exempt from colorant certification.[58]

Related to the characteristic blue coloration of phycocyanin are the innate fluorescent properties of the chemical. In particular, phycocyanin has been found to absorb light at 620 nm while emitting light at 640 nm.[35,59] This property has been found to have far reaching significance in several fluorescence-based applications, which are summarized in Table 1. A comprehensive review of the supporting properties of the phycocyanin molecule contributing to the current interest in fluorescent applications has been compiled by Glazer.[52]

Phycocyanin sensing is a potential detection method for measuring growth of cyanobacteria cultures and detecting cyanobacteria population in drinking water or native environs.[42,60] This aspect of phycocyanin has become increasingly important for water quality initiatives, as cyanobacterial blooms have been associated with toxic metabolite accumulation as well as drinking water sensory quality degradation. Through the collection of fluorescent

light absorbance data corresponding with phycocyanin concentrations, cyanobacterial blooms may be detected before populations have grown to dangerous or detrimental levels.[61,62]

The applications that have reached commercial scale include nutraceuticals, food and cosmetic colorants, and fluorescent probes for cellular and molecular detection.[60] The overall phycocyanin market value has been estimated at U.S. $ 10–50 million annually, with the price of phycocyanin ranging between U.S. $ 130–5,000 per kg depending on the grade.[63–65] With the cost of dry algal biomass alone estimated at U.S. $ 15–25 per kg, the significance of optimizing systems for increased phycocyanin productivity is crucial in maximizing the economic efficiency of algal production systems. Further expansion of the current phycocyanin markets may be possible as the bioeconomy expands and new opportunities for exploitation of its nutraceutical, colorant and fluorescent functionalities are found.

Phycocyanin Production Parameters

Current research on the microalgal production of phycocyanin has focussed on the establishment of optimal culture conditions. In the development of phycocyanin production systems for commercialization, such research may be used as benchmark for scale-up. Crucial parameters for phycocyanin production and their optimization strategies are reviewed in this section. Table 2 summarizes the system conditions where optimal phycocyanin productivity is achieved and the maximal transient productivities obtained (which ranged from 0.005 to 0.86 $g \cdot L^{-1} \cdot d^{-1}$).

Trophicity and Symbiosis

The trophic mode of the system is of particular significance in establishing a phycocyanin production system. Trophicity stands for the relationship of different organisms in an ecological community with the food resources in that system. Photoautotrophic

cultivation with *A. platensis* currently dominates the phycocyanin production market.[78] This is a product of the ease of biomass production rather than an optimization of phycocyanin productivity. *A. platensis* is generally cultivated axenically in common open-pond systems under natural sunlight and is a preferred species due to its proliferation in extreme pH of up to 10.5.[79] This characteristic is important as CO_2 absorption from the atmosphere is promoted at higher pH, which also helps maintain a monoalgal culture. This alone, however, does not constitute optimization of phycocyanin productivity. Rather, for particular species, mixotrophic production that combines the sum of photoautrophic and heterotrophic productivities can offer an increase in overall system's phycocyanin productivity, albeit with increased system complexity. For instance, a mixotrophic system is dually limited, in that it is simultaneously limited by either low or high light intensity (which affects the photoautotrophs) and by either low or high concentration of organic carbon substrate (which affects the chemoheterotrophs).[80] Consequently, stricter process control measures are required to maintain optimal productivity.

Previous research analyzing solely heterotrophic cyanobacteria cultivation had shown subpar results. Marquez et al. noticed significantly reduced concentrations of the pigments chlorophyll-a (–48%), carotenoids (–56%) and phycocyanin (–53%) under heterotrophic conditions compared to autotrophic conditions.[81] Trabelsi et al. studied the production of biopolymer (in the form of extracellular polymeric substances (EPS)) from *A. platensis* and found the highest specific productivity (433.62 $mg \cdot g^{-1} \cdot d^{-1}$) under photoautotrophic culture, while heterotrophic culture yielded the lowest specific EPS productivity (38.33 $mg \cdot g^{-1} \cdot d^{-1}$).[82] This result was attributed to the conversion of organic substrate into biomass rather than EPS under dark conditions.

Yet, recent studies with the rhodophyta species *Galdieria sulphuraria* have sparked renewed interests in heterotrophic production. An important development made was

overcoming relatively low specific phycocyanic concentration in the cells (3–4 $g \cdot kg^{-1}$ (dry weight, DW) for *G. sulphuraria 074G* versus 60–74 $g \cdot kg^{-1}$ (DW) for *A. platensis*) by means of high rates of biomass production that heterotrophic species provide.[74] Particularly, Graverholt and Eriksen were able to achieve the highest recorded phycocyanin productivity of 860 mg/L/day culturing *G. sulphuraria* heterotrophically.[74] To achieve this, a high rate of biomass production at high biomass concentration (83.3 $g \cdot L^{-1}$ (DW)) was used in continuous-flow culture mode, together with a strain having relatively high specific phycocyanin concentration (15.6 $mg \cdot g^{-1}$ (biomass DW).

The development of a microalgal polyculture system has also been suggested.[83] Such systems would take advantage of the diverse range of optimal growth conditions and metabolic mechanisms found among microalgal species by selecting species known to produce high-value metabolites such as phycocyanin. An economically sound system may be achieved as such strategy effectively increases the system's capacity for phycocyanin generation. This approach to microalgal cultivation has been slow to disseminate in the research community because there is such a wide variety of metabolisms developed by microalgae as observed in nature, so the use of a cultivation method based on mixed species with different specific metabolic capabilities requires extensive investigation to explore all possible combinations of species.[83] The benefits are evident, however, since the polyculture of mixed microalgal species, combining species with different metabolic abilities (e.g. the use of different forms of N, C and contaminants) may improve the overall production or phycoremediation capacity of cultures when supplemented with multiple or varying nutrient resources.[83]

Costa et al. examined the simultaneous cultivation of *A. platensis* and *Microcystis aeruginosa*.[84] It was found that the highly toxigenic *M. aeruginosa* did not have an effect on the growth of *A. platensis*. This could lead to reduced water supply costs for a microalgal

bioreactor; the cited study proposed using lagoon water as the main portion (80 vol%) of the culture medium. Currently, bioreactor water supplies are monitored and filtered to remove toxic bacteria. By eliminating this process and encouraging the symbiotic growth of the microalgae, this cost can be reduced or eliminated. Symbiosis stands for the close relationship between two species living in an ecological community. Another possible benefit of such system comes from the toxic metabolite production of *M. aeruginosa*. These compounds act as barriers to other organisms and prevent system contamination; this constitutes a commensalistic symbiosis (where one member of the community benefits while the other is not affected). Recent research has demonstrated that cyanobacterial toxins can function as algaecides, herbicides, larvicides and fungicides.[85] This has an added value to symbiotic phycocyanin production: the toxins generated can be removed as additional products, providing an opportunity for further economic benefit of the overall manufacturing process. Caution must be exercised, however, since cross-contamination of food-grade phycocyanin with toxic metabolites would pose a safety risk to the consumer. In these cases, highly effective harvesting processes are required to ensure complete separation, or perhaps preferably, co-culturing toxigenic microalgae should be limited to the production of phycocyanin for industrial applications.

Lighting Conditions

The optimization of photoautotrophic microalgae growth and metabolite production is significantly affected by lighting conditions, as the organisms utilize energy from light sources to drive metabolic activity. The light-harvesting pigment phycocyanin is directly linked to the photosynthetic processes of microalgae. Discussed within this section are numerous studies that have examined and confirmed the critical effect of lighting parameters

on phycocyanin production. Thus, comprehension of lighting condition relations can be applied to the improvement of overall system phycocyanin productivity.

Light Intensity

Research has shown that light intensity has a significant effect on microalgal phycocyanin production. Chen et al. determined that phycocyanin productivities in the cyanobacteria *Arthrospira platensis* increased to a maximum at 700 μmol/m^2/s photon flux density, due to an observed light intensity inhibition of cell growth above this level.[66] Takano et al. analyzed the effect of varying light source intensity on phycocyanin productivity of *Synechococcus sp. NKBG 042902*, and measured a maximum productivity at a photon flux density of 55 μmol/m^2/s at the surface of the culture vessel.[77] It is notable from Table 2 that the optimal light intensity even for a single species varies widely: optimal values for *A. platensis* vary from as low as 15 μmol/m^2/s to as high as 3000 μmol/m^2/s. It is unclear from the experimental methodologies why these values are so different; most works report the use of LI-COR quantum sensors, so measurement method does not appear to be the culprit. What is notable from Table 2 is that studies that report a higher optimal light intensity also report, generally, higher maximal transient phycocyanin productivities; so at least the two parameters appear to be linked: higher intensity results in higher productivity. It may signify that those studies that achieve higher productivities have better optimized other culture parameters and photobioreactor design.

Light Source Emission Spectra

Each photosynthetic pigment is adapted to absorb light within a specific range.[86] The spectral absorbance and fluorescence emittance wavelength maxima of phycocyanin have been determined as 620 nm and 640 nm, respectively.[35,59] It has been found that manipulation of light source emission spectra significant impacts both phycocyanin purity and productivity. Walter et al. found that applying a red-colored light filter to a *A. platensis* photobioreactor

system resulted in the highest phycocyanin purity as well as productivity per unit light intensity when compared to unfiltered, blue-filtered, and yellow-filtered light.[67] Similar results were achieved by Rodriguez et al.[87] A study on the effect of light quality on Synechococcus *sp. NKBG 042902* phycocyanin production determined that a range of 620-725 nm light source resulted in the greatest phycocyanin productivity when compared to broad spectrum light sources of emission wavelength maxima of 450-575 nm and 450-650 nm.[77] The maximal phycocyanin productivity determined by these experiments predominantly corresponds with absorption spectrum of phycocyanin.

Climatic and Nutritional Conditions

When optimizing microalgal phycocyanin production, attributes of the local climate must be taken into consideration. The local temperature and water source pH have been found to pose significant effect on microalgal phycocyanin production. Moreover, productivity is significantly affected by the nutritional contents of the growth media.

Temperature

The optimal temperature range for phycocyanin production is highly dependent on the microalgal species, due to the extensive variation of native climate. Numerous studies on the relation of temperature to phycocyanin productivity and purity for species native to warm climates have been conducted.[75,81,88,89] For *A. platensis*, *Arthronema africanum*, and *Anabaena albufera*, the optimal temperature for phycocyanin productivity has been determined to be 35 ºC.[87–89] Kenekar and Deodhar found that phycocyanin concentration of *Geitlerinema sulphureum* peaked at 25 ºC and started decreasing above that, despite increasing biomass productivity.[75] Although these temperatures correspond with temperate climates exclusive to specific geographies, phycocyanin producing microalgae are known to proliferate in both extreme heat and cold. The thermophilic cyanobacteria *Synechococcus*

lividus produces thermally-stable phycocyanin (which preserved light absorption functionality at 70 °C and became only slightly hypochromic at 80 °C) while maintaining growth at temperatures of up to 73 °C.[90] Conversely, arctic acclimated cyanobacteria (*Scytonema spp.* and *Phormidium spp.*, among other subdominant species) have had phycocyanin production identified.[91] Table 2 summarizes the temperatures used for optimal phycocyanin productivity. Notably, the study that reported the highest transient phycocyanin productivity (0.86 $g \cdot L^{-1} \cdot d^{-1}$) also utilized the highest temperature of the set (42 °C), but for the several studies that used 30 °C, the productivity values varied widely: 0.005–0.13 $g \cdot L^{-1} \cdot d^{-1}$). Therefore, higher temperature does not correlate conclusively with high productivity. Also, it should be noted that optimal temperature alone does not ensure optimal productivity, but rather multi-parameter optimization is required.

pH

The pH of culture media is a critical factor in maintaining optimal algal metabolism. Research has shown that variations in media pH may have an effect on phycocyanin productivity. Ogbonda et al. studied the influence of pH variation on *Arthrospira sp.* growth and composition, concluding that maximized protein productivity was achieved at pH 9.0, noting that the solubility of CO_2 and other mineral compounds is affected by pH; however no explanation was given for the subsequent decline in productivity at even higher pH.[92] It can be noted from their results, however, that higher productivity of crude protein correlated well with lower productivity of carbohydrate and crude fiber. Similarly, Morales et al. determined an optimal pH of 8.0–9.0 for phycocyanin productivity for a semicontinuous culture of *Anabaena sp. PCC 7120.*[73] According to Markou et al., an excessive rise of the medium pH can affect negatively the growth of microalgae either by the alkaline environment itself or due to low useful carbon availability, as carbonate (CO_2^{-3}) gradually becomes the dominant dissolved inorganic carbon form available (as opposed to bicarbonate at pH ~6.5–10.5) and

only some extremely alkaliphilic cyanobacteria can utilize carbonate.[93] It should also be noted that the cyanobacterial amenability to extreme temperatures can be analogously extended to viable pH ranges. For example, *G. sulphuraria* and *A. platensis* are two promising cyanobacteria species for phycocyanin production known to grow in media at pH 2 and 9, respectively.[66,74] This could be exploited in phycocyanin production systems to enable microalgal proliferation using a local water source with minimal pH adjustment.

Media

The production of phycocyanin in microalgal bioreactor systems occurs through a process of biosynthesis of metabolites in substrate media. The abundance of phycocyanin-producing cyanobacterial species coincides with a diverse variation of native substrates. Nonetheless, the significance of the media's composition is uniform regardless of species. Control of nitrate, phosphate, dissolved CO_2, and overall salt concentrations in substrate media have been demonstrated as essential for the optimization of phycocyanin production.[76,94]

While acting as a pigment-protein for the photosynthetic antennae of cyanobacteria (phycobilisomes), phycocyanin also functions as a nitrogen-storing component. According to Jin et al., nitrate (NO_3^-) is consumed more quickly than the other medium constituents, and thus becomes a limiting factor at an early stage of cultivation.[94] This has also been observed with algal blooms in nature, where nitrate becomes the primary limiting nutrient for algal proliferation in surface water.[94]

Research has found that the substrate nitrate concentration and source are key factors in regulating phycocyanin productivity. Chen et al. found that maximum phycocyanin productivity for *A. platensis* is achieved with $NaNO_3$ concentration of 0.045 M.[66] Also, the degradation of phycocyanin is associated with the depletion of substrate nitrate, as phycocyanin is utilized as a secondary nitrogen source in the absence of nitrate. Similar

findings were reported by Szalontai and Csatorday for the cyanobacteria *Anacystis nidulans*.[95] Kenekar and Deodhar determined that phycocyanin productivity of *G. sulphureum* increased by four times when increasing $NaNO_3$ concentrations from 1.5 up to 4.5 g/L.[75] These findings concur with the observed functionality of phycocyanin as a nitrogen storage compound in microalgae.[66]

A further attribute affecting phycocyanin productivity of cyanobacteria is the type of chemical used as the nitrogen source. Research has shown that the use of ammonium (NH_4^+) rather than nitrate as the nitrogen source for the growth of *Agmenellum quadruplicatum PR-6* and *Calothrix sp. strain PCC 7601* resulted in higher phycocyanin concentrations.[96,97] This effect was correlated by De Lorimier et al. to a higher cpcBA/apcAB RNA ratio detected in ammonium-grown cultures compared to nitrate-grown cultures, at a given irradiance.[97] Liotenberg et al. postulated that the changes in biliprotein content (phycocyanin versus phycoerythrin) are a means of adjusting the photosynthetic activity, in such a way that the production of reducing power does not exceed its demand.[96] Because ammonium-N is assimilated directly, whereas nitrate-N must be reduced (from +5 to –3 oxidation state),[97] the reducing power demand in lower in the case of ammonium-grown cells.

Markou et al. developed a unique approach for supply of ammonia (NH_3) from wastewater, whereby a natural zeolite (clinoptilolite type) was used as medium for the sorption of ammonia from wastewater and subsequently as nitrogen releaser in cultures of *A. platensis*.[98] According to Liotenberg et al., when externally supplied, ammonium ions enter the cells via an active transport system, while the unprotonated form (ammonia) enters by diffusion and is trapped by protonation.[96] The dependence of phycocyanin productivity on media nitrogen availability is a critical consideration in overall system design, and in some cases intermittent nitrogen feeding methods (e.g. fedbatch mode)[94] may have to be used to sustain productivity.

Phosphorous is another important nutrient for microalgal growth, present as a component of several organic molecules such as nucleic acids (RNA and DNA), membrane phospholipids and ATP, and thus making up 0.05–3.3% of biomass.[93] Phosphorus is frequently a limiting nutrient for microalgae, especially in natural environments, and can be found in various forms such as orthophosphate, polyphosphate, pyrophosphate, metaphosphate and their organic forms.[93] Depraetere et al. studied the effect of phosphate concentration of the growth of *A. platensis*.[99] That study was particularly concerned not to remove phosphate when treating piggery wastewater for color removal, as that would adversely affect the subsequent microalgal cultivation. Chitosan application resulted in >90% color removal with <20% phosphate (PO_3^{4-}) removal. Upon culturing in media with initial PO_4-P concentration of ~12–13 $mg \cdot L^{-1}$, biomass yield of *A. platensis* was nearly 50% higher in the chitosan decolored treatment than in the control treatment that was not decolored. Markou et al. used P-loaded zeolites (at 10.3 mg-P per g of zeolite) that supplied PO_4-P concentrations of 1.55–51.5 $mg \cdot L^{-1}$ to cultures of *Chlorella vulgaris* and *A. platensis*, with adequate growth being achieved for both species at the highest concentration.[100]

Carbon dioxide (CO_2) is an integral part of metabolic processes in photoautotrophic cyanobacteria. This concept has led to studies to determine the relation of CO_2 and phycocyanin productivity. Morales et al. concluded that the addition of 0.03% CO_2 (i.e. air) in a semi-continuous culture of *Anabaena sp. PCC 7120* resulted in significantly greater phycocyanin concentrations than 5% CO_2 addition.[73] The pH of the medium remained relatively low in the latter case (7.4 –7.8) compared to the former (which increased over time from 7.8 to 10). Similar results were achieved by Zeng et al., where it was determined that intermittent 20 $mM \cdot L^{-1} \cdot d^{-1}$ of CO_2 diluted in continuous 0.1 $L \cdot min^{-1}$ of air corresponded with the highest phycocyanin productivity in a *A. platensis* culture.[68] In this case, the added CO_2 attenuated the pH, due to its acidification effect, preventing it from surpassing 10 and

stabilizing it around 9. The reported phycocyanin productivity was lower (by 16–26%) when the culture was supplemented with the same flow rate of CO_2 or air alone. These results demonstrate that the moderation of CO_2 addition to bioreactor phycocyanin production systems is essential for optimization.

Further research has been conducted to analyze the effects of sodium bicarbonate and sodium carbonate additions to culture media on cyanobacteria phycocyanin productivity. Kenekar and Deodhar determined that phycocyanin productivity of *G. sulphureum* increased with increasing sodium bicarbonate concentration (between 0 and 10 g/L).[75] Bicarbonate acts as a pH buffer. Also, it was determined that a sodium carbonate concentration of 6.24 g/L resulted in the maximum phycocyanin productivity, 2.6 times higher than in the presence of 10 g/L bicarbonate alone.

A statistical analysis on media composition optimization for increased phycocyanin production from *Phormidium ceylanicum* was accomplished by Singh et al.[76] The concentrations of the four major components of BG-11 medium, namely $NaNO_3$, $CaCl_2{\cdot}2H_2O$, citric acid stock and trace metal mix, were optimized, at fixed concentrations of K_2HPO_4 and $MgSO_4{\cdot}7H_2O$. It was determined that citric acid addition had a beneficial effect on phycocyanin productivity concurrently with increasing nitrate concentration. An approximate optimal ratio of citric acid stock (composition per 100 ml: 0.06 g citric acid, 1 mg EDTA, 0.06 g ferric ammonium citrate, 0.4 g anhydrous sodium carbonate) to sodium nitrate concentration was found to be 30 ml/L:4 g/L. Also, the optimization of trace metal concentration to nitrate ratio was determined to be approximately 0.9 ml/L of trace metal mixture (composition per 100 ml: 0.286 g H_3BO_3, 0.181 g $MnCl_2{\cdot}2H_2O$, 0.022 g $ZnSO_4{\cdot}7H_2O$, 0.039 g $Na_2MoO_4{\cdot}2H_2O$, 0.005 g $Co(NO_3){\cdot}6H_2O$, 0.008 g $CuSO_4{\cdot}5H_2O$) to 4 g/L sodium nitrate. Overall, this study concluded that through an application of statistical media composition optimization, a phycocyanin productivity increase of 2.3-fold was

achieved compared to the original BG 11 medium, which was originally developed for isolation of freshwater blue-green algae.[101]

Operating System

Current methods of production for phycocyanin-containing microalgae include open-pond and closed bioreactor systems. Open-pond systems constitute the traditional method of microalgae production as well as the most common method of industrial microalgae production today.[102] This may be seen as a result of their relatively low investment cost and simplicity of design. Due to the open and uncontrolled nature of open-pond systems, however, their design is not amenable to optimization of phycocyanin productivity. Rather, the local environment of the pond strongly determines the phycocyanin productivity. A particular issue stemming from the open nature of these systems is the elevated risk of microbial contamination. This can have negative impacts on various aspects of the phycocyanin production, such as a reduction in available media, decreased phycocyanin purity, introduction of unfavourable symbiotic relationships, or introduction of toxic compounds that require further processing for removal. Other limitations of open-pond systems include: poor light utilization, inefficient temperature control, and susceptibility to substrate concentration variation due to evaporative losses, CO_2 desorption and inefficient media mixing.[103] Current research has shown maximum phycocyanin productivity levels for open-pond systems to range between 3-24 $mg \cdot L^{-1} \cdot d^{-1}$.[104–106]

Contrary to open-pond systems, closed bioreactor systems are intricately controlled to maintain optimal growth conditions. Research has shown that commonly controlled parameters of the closed-bioreactor systems also have an effect on phycocyanin productivity. The adoption of closed bioreactor systems has resulted in improved phycocyanin productivities, reported at levels ranging between 64-860 $mg \cdot L^{-1} \cdot d^{-1}$.[18,66] The potential of

several types of closed microalgal bioreactor systems has been analyzed at pilot-scale. Bioreactor designs of particular interest include flat-plate and tubular type systems. Both systems provide improvements over the traditional open-pond system through enhanced light source efficiency by increased surface area to volume ratio, elevated CO_2 retention, and reduced contamination risk. Ultimately, these benefits have been proven to allow for far greater culture population densities and phycocyanin productivity.[66,103,107]

Table 3 summarizes reported bioreactor operational parameters whereat maximal phycocyanin productivity was achieved. A primary element in the design of bioreactor systems is the operational mode. A comparison may be drawn from recent research utilizing varied batch, fed-batch and continuously operated microalgae production systems. Batch mode has been the most common configuration used in the research domain, followed by fed-batch. Batch systems are the least amenable to increasing phycocyanin productivity due to a lack of reactor content concentration control. The nature of batch systems of adding all media components at the beginning of culture growth is the source of the issue. This attribute of batch systems has been demonstrated to produce detrimental effects on phycocyanin productivity.[70]

Fed-batch systems eliminate the issues present in batch systems by stepwise additions of substrate, inoculum and other contents into the bioreactor. This method of addition corresponds with increased steadiness of optimal production conditions.[108] Literature has demonstrated the beneficial attributes of fed-batch systems in increased phycocyanin production in microalgal systems. Chen and Zhang found that overall phycocyanin content increased by 2.8-fold when comparing fed-batch and batch *A. platensis* bioreactors cultures.[70] Other research with *A. platensis* production has found that fed-batch systems allow for a 2.7-fold increase in biomass when compared to batch systems.[109] Although a fed-batch operating bioreactor offers increased constituent concentration control, a lack of control transpires

throughout downtime between additions. Continuous systems are an alternative operational mode for bioreactors that minimize this issue, although these require more research for use in microalgal phycocyanin production.

The highest phycocyanin productivity reported in recent research (Table 3) was achieved by culturing *Galdieria sulphuraria* in a continuous bioreactor.[74] This was possible because the continuous-flow culture enabled constantly high biomass concentration and the relatively high specific growth rate. Other research has demonstrated many cyanobacterial species with favourable amenability to continuous bioreactor production including *A. platensis*, *Phormidium bigranulatum* and *Microcystis spp*.[110–114] Continuous systems are characterized by constant monitoring and adjusting of reactor contents. Although this may maximize phycocyanin productivity, the resource investment for such a system would be substantial due to a lack of current technology. In the design of a microalgal phycocyanin production system, thorough analysis is required to determine optimal operational mode for both productivity and economics. In addition to bioreactor optimization, consideration must also be given to other steps in the production systems, such as separation, drying, extraction, etc.

Experimental reactor volumes have ranged in size from 250 ml to 50 litres, with mixing rates varying from stationary to 500 rpm (Table 3). Mixing was achieved either by gas sparging alone, rotary shakers or with the use of impellers; gas sparging, together with suitable reactor geometry, is favourable for lower operating cost. Forced aeration in photobioreactors was mainly done with sterile air, while experiments performed in flasks typically did not use forced aeration. Maximal phycocyanin productivity was achieved in batch systems after several days, while the continuous reactor was able to sustain high productivity with a residence time of 1.8 days (= 2.5 L/(1.38 L/day)).

An issue introduced by the closed nature of photobioreactors is the increased O_2 retention as a product of photosynthesis. Many algal species cannot tolerate oxygen levels above normative air saturation. Furthermore, increasing O_2 concentrations have been found to decrease photosynthetic efficiencies of *A. platensis*, which may result in decreased phycocyanin productivity.[107] To alleviate this, specific mixing regimes have been suggested. These mixing systems aim to provide adequate O_2 removal and culture distribution while limiting shear stress that may potentially damage the microalgae cells. Tredici and Materassi proposed the use of vertical alveolar panels, where mixing and deoxygenation of the culture suspension are effected by continuously bubbling air in the bottom of the panel.[115] They also noted that in serpentine design photobioreactors, the maintenance of oxygen concentrations compatible with growth of the selected algal species requires the installation of too many degassing stations, making the design impractical and non-economical. Comparatively, closed photobioreactors have so far seen little adoption in the industrial production of microalgae and phycocyanin due to the long road for technological development and the accompanying large capital costs.

Phycocyanin Production Modelling

To further the comprehension of microalgal phycocyanin production mechanism, predictive growth models have been proposed in literature. Currently identified models have commonly correlated variables of substrate nutrient concentrations, light intensity, biomass concentration, and particular product concentrations. Chojnacka and Noworyta have documented a summary of models for the growth of photoautotrophic microorganisms from literature.[116] A further resource by Yuan et al. provides a consolidation of microalgae models investigating the effect of additional system parameters.[117] Such models may be applied to photobioreactor cultivation of microalgae to determine system conditions required for

optimal phycocyanin productivity. The benefits of microalgal population growth models include: (i) the establishment and maintenance of proper system cellular population density; (ii) the determination of optimal system parameters through an economically sound approach; (iii) aiding the upscaling of bench top systems to larger commercial manufacturing facilities.

Table S1 in the Supporting Information summarizes established literature models of microalgal phycocyanin production. Most models are mechanistic in nature and take the Haldane equation into account to simulate substrate inhibition effects. The one notable exception is the model proposed by Singh et al. (Equation (1)), which is based on Response Surface Methodology.[76] It is a statistical modelling technique whose objective is to determine the optimal operational condition for the system or to determine a region that satisfies operating specifications. An experimental program utilizing central composite design in needed to calibrate the model. The authors were able to increase phycocyanin productivity from *P. ceylanicum* by 2.3-fold by finding the optimal medium composition, and found good agreement between predicted and experimental values (R^2 = 94.00, signal to noise ratio = 17.964).

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \cdots \quad (1)$$

Tables S2a and S2b in the Supporting Information summarize established literature models of cyanobacteria biomass production. Main differences between the models include taking light intensity into account for photoautotrophic species, and considering either carbon or nitrogen as the limiting substrate. Chen et al. investigated the effect of LED color on growth kinetics, but their model equation (Equation (2)) does not have a term for light wavelength or intensity; rather, color and intensity are taken into account through the data-fitted constants μ (maximum biomass concentration) and X_m (initial specific growth rate).[71]

Red light gave the best performance, and blue the poorest; values of μ and X_m at the highest intensity (3000 μmol·m^{-2}·s^{-1}) were, respectively, 2.0 and 7.2 times greater for the former.

$$X(t) = \frac{X_0 e^{\mu t}}{1-\left(\frac{X_0}{X_m}\right)(1-e^{\mu t})} \quad (2)$$

Additional models have been established to analyze the metabolic aspects of microalgal systems in their native ecosystems. Guven and Howard compiled a comprehensive review of mathematical and artificial neural network models of cyanobacterial growth in their natural freshwater habitats.[123] Such information is applicable to microalgal phycocyanin production systems emulating optimal natural habitats. The isolation of a microalgal species from a model-predicted bloom will be an ideal candidate for the axenic species of a freshwater habitat-emulated bioreactor system. Native marine habitat growth modelling of phycocyanin-producing microalgae has also been researched. The symbiotic growth of cyanobacteria, purple and colourless bacteria in marine microbial mat communities has been analyzed and modelled by Wit et al.[124] This work consolidates multiple models that take into account different species, metabolic types, and environmental parameters including oxygen concentration, total free sulfide, photosynthetic active radiation and near infrared radiation. Such a model may prove applicable to bioreactor systems opting for a polyculture environment with marine species.

The development of models that describe the metabolic ability of microalgae to remove nutrients from waste waters is pertinent to the environmental impact of microalgal cultures. Such models hold particular significance to phyco-valorization systems by allowing a further comprehension of a culture's capacity to utilize waste effluents. This information may in turn be used to determine optimal photobioreactor parameters for commercial

production systems. A summary of models analyzing nutrient uptake by phycocyanin-producing microalgae is shown in Table S3 in the Supporting Information.

The most prevalent substrates modelled are nitrogen and organic carbon compounds. Most models utilize mass balance or reaction kinetic equation, and some account for substrate autoinhibition with Monod-type terms. Lu et al. present a unique model that accounts for the mass transfer process and the growth kinetics of alginate-entrapped cyanobacteria *Anabaena CH3* in a batch reactor (Equations (3) and (4)), to predict the removal of nitrogenous compounds in wastewaters.[121] The governing equations were cast in dimensionless form and solved by the method of explicit finite difference. The model could capture the mass transfer behaviour around a spherical bead and the diffusion process within the cell-containing gel. Lu et al. noted that immobilized microalgae could overcome some difficulties found with the application of suspended microalgae for wastewater treatment, namely maintaining monospecificity and separation of biomass from effluent before discharge.[121] The challenge with immobilized cells however, is that these cells experience lower substrate concentrations within the gel (modelled by Equation (3)) than those in the bulk liquid (modelled by Equation (4)), because of diffusion limitations, which hinders the substrate consumption (and hence wastewater decontamination) rate.[121] Dang et al. focussed on iron uptake rates (Equation (5)), as cyanobacteria have a relatively high iron requirement to sustain processes of photosynthetic and respiratory electron transfer and, in some cases, nitrogen fixation.[125] The study found that the rate of iron uptake was lower for cells grown under conditions of lower iron availability, suggesting that cells adjusted by decreasing their maximum uptake rates (ρ_{max}) while maintaining a constant affinity (K_p) for iron.

$$\frac{\partial N_g^*}{\partial t^*} = \left(\frac{\partial^2 N_g^*}{\partial r^{*2}} + \frac{2}{r^2}\frac{\partial N_g^*}{\partial r^*}\right) - \phi^2 \frac{N_g^*}{1+N_g^*} \quad (3)$$

$$\frac{\partial N_b^*}{\partial t^*} = -3\alpha \cdot Bi(N_b^* - N_s^*) \quad (4)$$

$$\rho_{Fe} = \rho_{max} \frac{[Fe(II)']_{ss}}{K_p + [Fe(II)']_{ss}} \quad (5)$$

The development of novel predicative models relating lighting parameters to the growth of cyanobacteria is an additional subject of current research interest. A comprehensive model combining the mechanisms of monodimensional radiative energy transfer by light and metabolic processes of *A. platensis* has been developed by Cornet et al.[69,127] These works focused on establishing a simplified method for determining the optimal parallelepipedic photobioreactor system parameters for microalgal growth, which allows for a further application to systems of more complex geometries. More recently, a model was developed by Gorbunov et al. that explored the mechanism of non-photochemical quenching in cyanobacteria.[128] Photobioreactor systems utilizing daylight as the illumination source will often be subject to extreme light intensities capable of damaging the culture. This model allows a further comprehension of the photoprotective mechanics employed by cyanobacteria under such conditions.

Phyco-valorization: Integrated Resource Recovery

The sustainable engineering practice of integrating a value recycling process within existing infrastructure is known as integrated resource recovery.[129–131] Oftentimes, the addition of such a process has both the benefit of environmental remediation and of increased system economics efficiency. Phycoremediation technology has a significant amenability to resource recovery from a variety of waste streams. The idea for phycoremediation stems from the observation that cyanobacterial blooms are often stimulated by the hypertrophication of natural habitats by means of human/industrial waste disposal.[132,133] Phycoremediation involves the integration of waste into bioreactors, where the microalgae acts to chelate metals, or to degrade unwanted compounds.[83] Potential resources drawn from unused

industrial waste streams or infrastructure include substrate sources, excess temperature control streams, pH adjusting materials, and available light.

A step further in the sustainable engineering practice is phyco-valorization: the exploitation of microalgae and microalgal chemicals as valuable products. Figure 1 illustrates an example from literature where flue gases are treated in photobioreactors, leading to the production of valuable algal products after a biomass processing stage. Here, the authors propose the use of extremophilic algae when using flue gases directly or after concentration of acidic compounds. These species not only tolerate extreme environmental conditions but require such conditions to thrive, which becomes a major advantage since these cultures are less affected by outdoor contamination.[65] Examples are the cultivation of thermophilic cyanobacteria or species growing in diluted sulfuric acid (i.e. acidophilic); the red algae *Cyanidium caldarium* and *G. sulphuraria* are both thermo- and acidophilic.[65] The process of microalgal product recovery involves separation of the algal biomass from the liquid medium, still containing nutrients that can be recycled, followed by extraction of the desired product from the biomass using techniques such as drying, extraction and esterification.

The harvesting of commercially viable components from the phycoremediation process has been suggested as one way to increase the feasibility of this technology.[28,134] Through optimizing the production of the high-valued pigment phycocyanin in a phycoremediation process, the process may be able to advance into a system of phyco-valorization, posing both opportunities for environmental remediation and increased profits for potential manufacturing facilities. This concept may also apply to systems where phycocyanin producing microalgae may be present although not encouraged, such as in water purification applications. Common to water quality programs is the detection of phycocyanin concentration due to its correlation with increased toxicity imparted by cyanobacteria.[61,62] In this scenario, the resources spent in detecting and removing phycocyanin-producing

microalgae may be recuperated through isolation of the available phycocyanin. Further research is required to evaluate the prospect of isolating phycocyanin from the water purification process in the event of a toxic and phycocyanin-rich cyanobacteria bloom.

Reaching Optimal Production Parameters via Phyco-valorization

In this section, the same parameters that were discussed as necessary to monitor and control for optimal phycocyanin productivity in dedicated systems are discussed again in the context of phyco-valorization. That is, how are these parameters controlled in a system that utilizes waste streams or residual resources to achieve the optimal (or near optimal) conditions of dedicated systems (summarized in Table 4).

Lighting Conditions for Phyco-valorization

When considering the development of a phycocyanin production system, the integration into a functioning venture can greatly improve overall efficiency. In this scenario, construction of a microalgal phycocyanin production bioreactor should be accompanied by a thorough analysis of available light sources. Alternatively, new manufacturing facilities can be designed to optimally integrate microalgal phycocyanin production with the other manufacturing activity (e.g. horticulture) in a way that maximizes overall efficiency.

Numerous industries utilize outdoor production facilities with ample natural sunlight covering, such as agricultural or concrete manufacturing plants.[135,136] Such production plants could take advantage of the light exposure by integrating a photobioreactor system into the existing infrastructure for algal phycocyanin production. This would be especially applicable to facilities producing waste effluents that could function in other areas of the bioreactor system such as medium and pH adjustment. Although natural daylight provides the greatest light intensity with wide ranging spectrum, attention should be drawn to its drawbacks. Sunlight reaches maximum intensity levels that have been shown to decrease cyanobacteria

production rates.[137] This may be due to the surface of the culture experiencing light intensities exceeding the optimal threshold.

Currently functioning artificial lighting system facilities is another option for photobioreactor system integration. Artificial lighting provides a readily controlled light source with constant intensity and spectrum. However, the excessive cost of powering artificial lighting systems has resulted in their limited use. Despite this, a number of industries currently benefit from the use of artificial lighting systems, such as indoor horticultural practices. One study reported that in the Netherlands, 19%, or about 2000 ha, of the total glasshouse area is equipped with supplementary assimilation light.[138] Literature has also suggested the beneficial effect of unifying microalgal production systems simultaneously with aquaponic systems, many of which utilize artificial lighting.[139]

The artificial lighting equipment used in industry has specific light intensity and spectrum corresponding with the particular production system. In horticultural practices, the growth response of plants to different wavelengths differs, and as such these artificial lighting systems are designed to maximize Photosynthesis Active Radiation (PAS). The selection of suitable lamps is also made in a manner that maximizes the Photosynthetic Photon Flux to wattage ratio (PPF/W); this measure is greatest for high pressure sodium lamps, and is lowest for incandescent and halogen lamps (GE Lighting, http://www.gelighting.com/LightingWeb/kor/images/Horticulture_Lighting_Brochure_EN_tcm563-12710.pdf). In one study on tomato culturing, it was found that high productivity requires dominance of the 600-700nm red portion of the irradiation spectrum.[140] The same study also reports that in cucumber culturing, blue or green irradiation alone do not promote development; rather a balance between blue, green and red lighting is required.

By adapting a microalgal phycocyanin production system to a facility with optimal artificial lighting conditions, a significant increase in lighting utilization can be achieved.

However, since the lighting conditions are optimized for other purposes, the microalgal cultivation system may not operate at its optimal lighting parameters (Table 2). Thus, the optimization of other process parameters that balance this shortcoming would be required.

Climatic and Nutritional Conditions for Phyco-valorization

Common to manufacturing facilities is the utilization of energy sources for heating and cooling duties.[141] These temperature control processes have led to the development of sustainable engineering design of heat recovery systems. Such systems aim to identify and take advantage of opportunities throughout the facility to reuse residual heat. Overall, the efficiency of the system is increased by reducing energy consumption.[142] An opportunity for heat recovery may be realized through the integration of a microalgal bioreactor. Many of the notable potential microalgal species have had maximal phycocyanin productivities recorded at increasingly warm temperatures (Table 2). Two of the most productive species, *A. platensis* and *G. sulphuraria*, have had their maximum phycocyanin productivity recorded at or above 30 ^{o}C. [66,74,89] Considering that only selected climates are able to maintain such conditions for suitable lengths of time, bioreactor temperature control is necessary to maintain optimal production rates. Continuous utilization of a residual heat stream to apply further control of the bioreactor temperature may result in increased spent energy value. An economic analysis of this energy recovery system is required on a case-by-case basis to determine viability.

Controlling photobioreactor media pH at or near optimal values (Table 2) is necessary to ensure the optimal culture growth and phycocyanin productivity. The use of highly alkaline or acidic compounds to adjust system pH is a commonly used method to achieve this control; however, significant costs are imparted. Thus, when developing a microalgal phycocyanin production system, more efficient measures of controlling pH should be explored. A primary consideration should be the integration of local untreated or waste-

derived water source as the basis of system media. This allows for overall reduction of pH control costs in two possible ways – the selection of a culture species amenable to the pH of the water source, or the selection of a water source with a pH requiring minimal adjustment. The wide range of viable pH levels for optimal microalgal growth (Table 2) may lend itself to the selection of an agreeable species.

Although adjustment of the bioreactor pH may be minimized through the use of suitable local untreated or waste-derived water, the production of algal metabolites throughout the process will ultimately drift the system pH away from optimal levels. Thus, an analysis of possible pH adjustment agents should be realized. The utilization of highly acidic or alkaline system effluents is a principal opportunity. The viability of recycling these streams into useful pH adjustment agents has been previously demonstrated; for example, Atkinson isolated organic acids using bipolar membrane electrolysis.[143] A microalgal phycocyanin production system that is able to maintain pH levels using an untreated effluent may provide a highly economic means of treating these waste streams, as such streams are often difficult to dispose of due to the magnitude of pH.

The significant cost of standard laboratory grade media formulations, along with the wide range of native substrates identified for phycocyanin-producing microorganisms, has propelled a search for economic media alternatives.[144] Wastewater and other waste effluents are highly economic potential media sources. Furthermore, while serving as media for microalgal phycocyanin production, the use of a waste effluent-based media brings the additional function of environmental remediation.

Current research has demonstrated the feasibility of using waste effluent from an assortment of industries as bioreactor substrate media. Vetayasuporn determined the viability of rice noodle factory-derived wastewater for use as substrate media for the growth of *A. platensis*.[145] Other food-based industrial effluents have also been identified as promising

nutrient sources, including dairy and brewery waste streams.[146–148] The use of agricultural swine wastewater as medium for *A. platensis* growth has also proven successful.[149,150] Similarly, aquaculture waste effluent has been identified as a potential microalgae media source.[139] A study by Dunn et al. found industrial tannery wastewater to be an acceptable substrate for *A. platensis* production.[151] The use of municipal wastewater as a viable media for microalgal growth has been demonstrated by numerous studies.[152–154] El-Bestawy confirmed that the cyanobacterium *Tolypothrix ceytonica*, *Anabaena variabilis* and *Anabaena oryzae* have adequate growth in highly polluted industrial-residential wastewater.[155] Caution should be exercised, however, when looking to produce food-grade microalgal products, as contaminants from the wastewater may pose a safety risk if not completely removed from the isolated final product.

A further waste source for phycocyanin production system media is industrial flue gas, which is known to contain high CO_2 concentrations, such as that from ethanol distillation,[83] and to also contain NO_x (which forms nitrate in solution, acting as additional electron acceptor[156]), such as power plant flue gases.[157] Recent research has shown that a variety of phycocyanin-producing cyanobacteria have favourable potentials for using industrial flue gas as carbon substrate source.[156–158] Arata et al. studied the possibility of simultaneously feeding CO_2 and NO_x gases during cultivation of *A. platensis*, and found best results when using opportune dosages in a fed-batch reactor.[157] Kumar et al. reviewed the literature on CO_2 sequestration in photobioreactors, and concluded that based on system requirements for high solids to volume ratio, mixing, mass transfer, scalability and ease of operation, an airlift reactor integrated with a tubular loop reactor is most promising option.[158] This hybrid design exploits the advantages of the two different type of reactor, and one overcomes the disadvantage of the other.[158]

Economic Evaluation of Existing Infrastructure Integration

The costs associated with novel microalgal phycocyanin production systems has been previously reviewed by Vonshak.[159] Adjusted for inflation, the capital cost of a pond system producing 40 tonnes of biomass annually was determined to range between U.S. $863,580 - $2,633,000 (in 1992 value). Similarly, the annual operating cost was determined to range between U.S. $419,116 - $1,754,205 (in 1992 value). Through the application of infrastructure integration, the costs can be greatly reduced. An economic analysis of a similar microalgal bioreactor-based production system determined media to constitute approximately 17.5% of the total operating costs.[160] Thus, a substantial reduction in production costs may be achieved through the use of waste effluent nutrient-based media and carbon dioxide derived from flue gas. Recycling thermal energy from other industrial processes can also allow for a significant reduction in production cost. Optimal temperature control of the system was determined to contribute 8% of the total operating costs in the economic analysis by Li et al.[160] Capital costs may be reduced through integrating the construction of a microalgal phycocyanin production facility. Manufacturing facilities amenable to such integration are those that could provide a means for the previously discussed operating cost reductions. Building area and land costs have been estimated to range from 8.9% - 30.6% of the capital costs.[159,160]

In order to provide a reliable economic evaluation of a microalgal phycocyanin production system, a comprehensive cost analysis is required. The procedure for such an analysis would entail the construction, operation, and evaluation of a large-scale pilot bioreactor system. The establishment of a pilot-scale operation may be accomplished applying growth models and optimal system parameters established in recent literature. Further optimization for a specific location and production volume may be accomplished by localized pilot system evaluation. This system would optimally be operated for multiple years

in order to determine the effect of seasonal environmental transitions. Upon system optimization, the established parameters can be scaled to larger pilot systems. The amount of pilot-scale testing is relative to the degree of reliability required for the final production system. Li et al.[160] demonstrate a comprehensive analysis of system parameters to be established for optimal commercial microalgae production.

Conclusions

Microalgae constitute a fundamental component of the biosphere through their contribution of nutrient and sunlight energy transforming mechanisms. As a means of nitrogen storage and light energy capture, the pigment phycocyanin is a necessary compound for numerous microalgae to continue their ecologic role. This innate function of microalgae may be exploited through the culturing of species amenable to industrial practice. The recent surge of interest in the application and production of microalgal phycocyanin has demonstrated the promising economic outlook of photobioreactor systems. In order to maximize the potential of such systems, several strategies to enhance phycocyanin productivities are advocated. A primary consideration of a microalgal phycocyanin production system is the species present in the culture. Beyond agreeing with local climate conditions, additional factors to consider include attributes of culture trophic mode as well as interspecies relations. Research has demonstrated that the phycocyanin productivities of various species are dependent upon the system lighting, climatic conditions, substrate component concentrations, as well as the bioreactor operational mode. Both previous research and further case analysis may be applied to balance the factors contributing to overall system productivity. An economically sound approach to achieving system optimization is the modelling of metabolic kinetics and subsequent scaling to desired production. Several models have been previously developed and may be used as benchmarks for this endeavour. A further approach for improving the

viability of microalgal phycocyanin production systems would be their application in integrated resource recovery. Previous efforts have determined the viability of microalgae in environmental remediation initiatives. Through the incorporation of phycocyanin productivity optimization principles into a phycoremediation process, the harvesting of phycocyanin may valorize otherwise wasted resources. The simultaneous economic potential and environmentally-forward concept of phyco-valorization through phycocyanin production is a promising application of microalgal biotechnology awaiting further development for industrial implementation.

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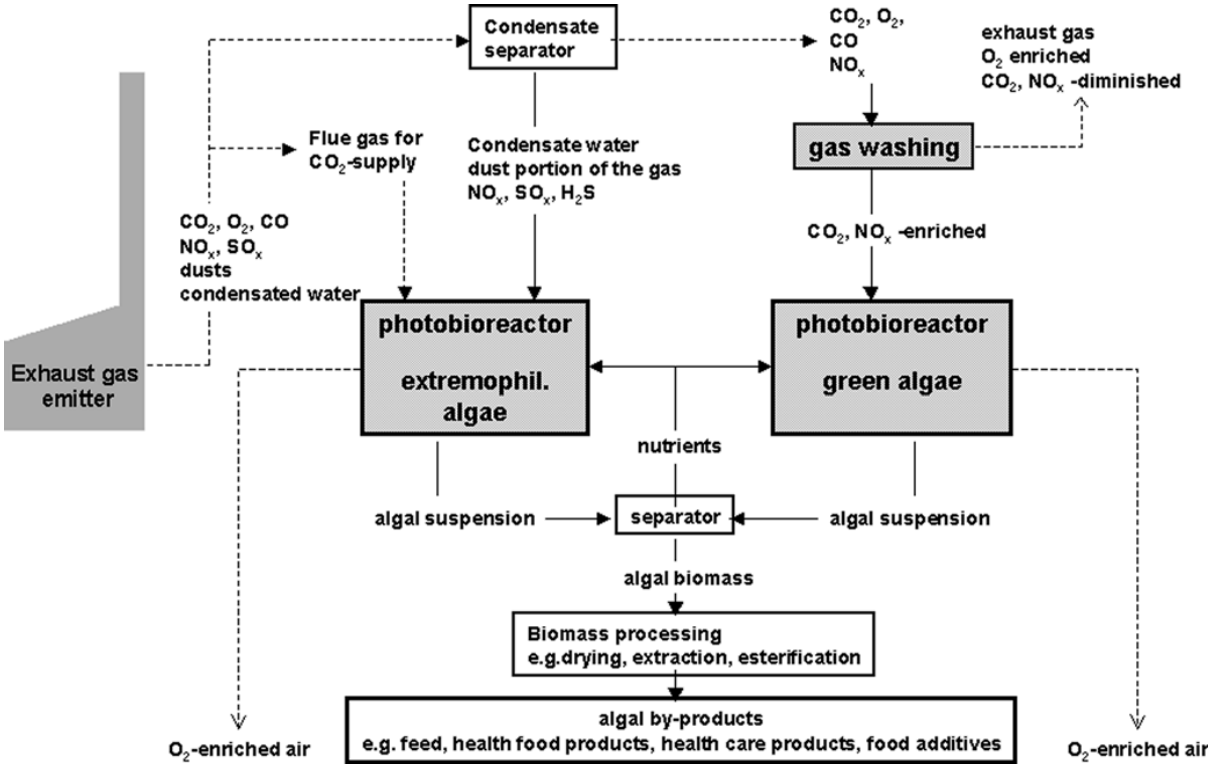

Figure 1: Scheme for CO_2 fixation from industrial exhaust gas by microalgae (Pulz and Gross[65], reprinted from Applied Microbiology and Biotechnology (2004), vol. 65(6), pp. 635–648, with kind permission from Springer Science and Business Media, CCC licence 3623800818054)

List of Tables

Table 1: Identified Nutraceutical Functions of C-phycocyanin and Applications of Its Fluorescent Properties

Nutraceutical Functions	Reference
Inhibits growth of leukemia cells.	[33]
Lowers blood lipid, combating fatigue and increases the level of immunoglobulin A (IgA) and immunoglobulin M (IgM).	[34]
Nephroprotection, antioxidant activity, metal chelation, neuroprotection.	[35]
Slows cancer progression, prevents atherosclerosis development, improves blood lipid profile, reduces allergic inflammation and protects against hay-fever.	[36]
Promising anti-fungal and anti-viral activity.	[37]
Potent free-radical scavenging ability.	[38]
Increases the expression of essential enzymes and biochemicals related to the balanced function of liver and kidney, leading to the improved detoxification.	[39,40]
Applications of Fluorescent Properties	**Reference**
Superior fluorescent dye for medical tagging.	[41]
Method of monitoring potentially harmful cyanobacterial populations in native environs.	[42,43,44,45,46,47,48,49,50]
Fluorescent label for immunoassay.	[51]
Use as fluorescent tags in histochemistry and reactive oxygen species assay.	[52]
Electrophoretic application in monitoring protein blotting and the focusing time of protein samples during isoelectric focusing.	[53]
Determination of phycobiliprotein composition of marine and freshwater picophytoplankton in oligotrophic environments.	[54]
Monitoring influence of protein encapsulation and studying matrix and protein interaction and stability of protein in silica matrix of Phycocyanin-doped silica biomaterials.	[55]
Fluorescence emitter in fluorescence-activated cell sorting flow cytometry.	[56]

Table 2: Summary of Optimal Phycocyanin Productivity by Microalgal Species and Respective Bioreactor Parameters

Species	Lighting Conditions			Climatic Conditions		Media		Phycocyanin Productivity	Reference
	Spectrum/Quality	Light Intensity	Light Cycle (day:night hours)	pH	Temp (°C)	Type	Modification	Maximal transient ($g \cdot L^{-1} \cdot d^{-1}$)	
A. platensis	Fluorescent white	700 $\mu mol/m^2/s$	*NR*	9	30	Unique	$NaNO_3$ 0.045 M (initial)	0.13	[66]
	Red filtered sunlight	800 lux (~15 $\mu mol/m^2/s$)	12:12	8.5-9.5	25.3	Diluted Zarrouks	Diluted to 20%	0.01[a]	[67]
	Fluorescent white	200 $\mu mol/m^2/s$	14:10	9	30	Zarrouks	None	0.07[a]	[68]
	Fluorescent white	8-18 W/m^2 (~37-83 $\mu mol/m^2/s$)	*NR*	9.5	36	NO^{3-} reduced Zarrouks	NO^{3-} 0.29 g/L	0.008[a]	[69]
	Fluorescent	160 $\mu mol/m^2/s$	24:0	9.5-10.5	30	Modified Zarrouks	0.5 - 2 g/L glucose	0.07[a]	[70]
	Red LED	3000 $\mu mol/m^2/s$	24:0	*NR*	30	Zarrouks	None	0.02[a]	[71]
	Fluorescent white	80-120 $\mu mol/m^2/s$	24:0	9.5	30	Modified Zarrouks	2 g/L glucose + 0.2 g/min glucose after 5th day	0.10[a]	[72]
Anabaena sp. PCC 7120	Fluorescent white	110 $\mu mol/m^2/s$	12:12	8 - 11	28	BG11 with HEPES buffer	20 mM HEPES buffer	0.02[a]	[73]
G. sulphuraria 074G	No light	No light	No light	2	42	Unique	50-150 g/L glucose + 1.38 L/day glucose sol'n, 50g/L NH_4 initial	0.86	[74]
G. sulphureum	Cool white fluorescent	1000 lux (~14 $\mu mol/m^2/s$)	16:8	8- 9	30	Modified Zarrouks	$NaNO_3$ 3.5 g, Na_2CO_3 6.24 g/L	0.005[a]	[75]
Phormidium ceylanicum	Fluorescent white	130 $\mu mol/m^2/s$	12:12	7.4	27	BG11	Optimized $NaNO_3$, $CaCl_3$, citric acid, trace metals.	0.02[a]	[76]
Synechococcus sp. NKBG 042903	Red LED (660 nm peak)	55 $\mu mol/m^2/s$	24:0	*NR*	25	BG11 with NaCL	30 g/L NaCL	0.023	[77]

NR: Information was not recorded in relevant literature

[a] *Calculated values*

Table 3: Summary of Bioreactor Configurations and Parameters for Optimal Phycocyanin Productivity

Operational Mode	Geometry	Agitation	Forced Aeration	Growth Period[b]	Microalgal Species	Maximal Transient Phycocyanin Productivity ($g \cdot L^{-1} \cdot d^{-1}$)	Reference
Batch	Flat-type (1 L)	300 rpm	2.5% CO_2 , 0.2 vvm , (80-100 um diffusion)	*NR*	*A. platensis*	0.13	[66]
Batch	Flat-type (500 mL)	Gas sparging	Air, 1 vvm	2 days	*Synechococcus sp. NKBG 042903*	0.023	[77]
Batch	Conical Flask (250 mL)	Continuous orbital shaker	None	15 days	*G. sulphureum*	0.005[a]	[75]
Batch	Open Tank (50 L)	*NR*	Air, 0.03 vvm	18 days	*A. platensis*	0.01[a]	[67]
Batch	Conical Flask (250 mL)	*NR*	None	32 days	*Phormidium ceylanicum*	0.02[a]	[76]
Batch	Custom Photobioreactor (~275 mL[a])	Gas sparging	20 mM/L/day intermittent CO_2 + 0.1 L/min continuous air	6 days	*A. platensis*	0.07[a]	[68]
Batch	Parallelepipedic (4 L)	*NR*	Air, $1.6x10^{-5}$ Nm^3/s	11.2 days[a]	*A. platensis*	0.008[a]	[69]
Batch	Conical Flask (500 mL)	120 rpm	None	4 days	*A. platensis*	0.02[a]	[71]
Continuous	Bioreactor (3 L)	500 rpm	2.5 L/min air	1.38 L/day	*G. sulphuraria 074G*	0.86	[74]
Fed-Batch	Conical Flask (250 ml)	*NR*	0.03% CO_2 , 45 µm filtered (flow rate *NR*)	10 days	*Anabaena sp. PCC 7120*	0.02[a]	[73]
Fed-batch	Fermentor (3.7 L)	300 rpm	Sterile air, 100 L/h	12.5 days	*A. platensis*	0.07[a]	[70]
Fed-Batch	Bioreactor (3.7 L)	300 rpm	Sterile air, 100 L/h	7 days	*A. platensis*	0.10[a]	[72]

[a] *Calculated values.*

[b] *Growth Period is recorded at maximal transient phycocyanin productivity.*

NR: Information was not recorded in relevant literature.

Table 4: Summary of Optimal Conditions for Phycocyanin Production in Dedicated Systems

• Mixotrophy (autotrophs + heterotrophs). • Polyculture (multiple metabolisms, symbiotic toxin-generating species). • High light intensity (up to system specific limit) with maximum emission wavelength near 620 nm. • Temperature dependent on native climate of species • Optimal pH is typically, though not exclusively, situated in the range where bicarbonate speciation of dissolved inorganic carbon dominates (i.e. <10.5). • Nitrogen supply in the form of ammonium, with care not to induce substrate inhibition. • Added CO_2 has limited effect, especially if acidification is not controlled. • Sodium carbonate additions are beneficial. • Citric acid supplementation to growth medium. • Continuous reactor, designed to use air sparging (possibly enriched with CO_2 up to few vol%) for mixing/circulation and for deoxygenation.