The “bright side” of cyanobacteria: revising the nuisance potential and prospecting innovative biotechnology-based solutions to integrate water management programmes

Inês P. E. Macário[1,2], *S. P. M. Ventura*[2], *F. J. M. Gonçal*ves[1], *M. A. Torres-Acosta*[3,4], *J. L. Pereira*[1]

[1]Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

[2]CICECO - Aveiro Institute of Materials & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

[3]The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 6BT, UK

[4]Tecnologico de Monterrey, Escuela de Ingenieria y Ciencias, Ave. Eugenio Garza Sada 2501 Sur, Monterrey, Nuevo Leon, 64849, Mexico.

Corresponding authors:
Ventura, S. P. M. spventura@ua.pt
Pereira, J. L. jpereira@ua.pt

Twitter: @PATh_RG

Synopsis:
Biotechnological exploration of cyanobacteria “bright side” can be linked with sustainable environmental protection.

Abstract. Global warming and the anthropogenic degradation of water quality are pointed out as main causes of the worldwide increase in frequency, severity, and duration of harmful algal blooms (HAB). Cyanobacteria, major constituents of HAB, can cause ecological, economic, and human health problems, configuring a “dark side” requiring management attention. Their growth can be potentiated by climate change consequences, highlighting further the urgency of improving HAB management strategies to ensure water quality. An innovative perspective for cyanobacteria management is the exploitation of their “bright side”. Several exploitable products produced by cyanobacteria (e.g. bioactive pigments, lipids, proteins) present high market value. Thus, this work provides a critical perspective on how HAB management may be connected with biotechnology in the future. We propose the use of the biomass of cyanobacteria blooms physically removed in traditional control actions (much needed to ensure environmental and even human health safety) as a feedstock for future valorization, thus allying profit to water quality management, in a win-win relationship between economics and sustainability. Such a proposal was validated with an economic analysis, which evidenced a relevant potential for a positive Return (hence rendering profit likely to occur), both considering only the delivery of harvested biomass to production units and the full valuation route from harvesting to the selling of the extracted/purified product using phycocyanin and chlorophyll as models, under a multi-product strategy.

Keywords: cyanobacteria blooms, water management, biomass exploitation, biotechnology, economic impact

1. Introduction

The warming of the climate system is unequivocal. Due to the changing in precipitation patterns or the melting of snow and ice, hydrologic systems suffer alterations in their common cycles, with consequences in water quantity and quality [1]. Under a global change scenario, water scarcity in many regions will became (or already is) a reality, and contamination of freshwater sources will continue to be an issue (e.g. 80% of the world's population already suffers serious threats on water security [2]). One of the threats to water degradation is the proliferation of phytoplankton groups such as cyanobacteria. Along with the anthropogenic eutrophication and increasing CO_2 levels in the atmosphere, climate change manifestations, namely rising temperatures and altered hydrologic patterns, are strong drivers of the increased frequency, intensity and duration of cyanobacterial blooms [3–5]. Thus, water management programmes need to increasingly consider these organisms to assure the human health and ecosystems safety. On the other hand, cyanobacteria have the biochemical potential to be commercially exploited in agriculture, aquaculture, bioremediation, biofuels, nutraceutical and pharmaceutical products [6–9]. This imposes the key question on whether exploiting cyanobacteria blooms can be a sustainable strategy supporting the management of affected waterbodies. Here, we document the suitability of exploring the nuisance biomass removed in environmental control actions and the economic income that can offset management costs. We first provide key definitions for the understanding of the field, regarding the ecophysiological advantages of cyanobacteria and how climate change promotes them. Then, the critical problem of bloom formation and cyanotoxins production configuring the "dark side" of cyanobacteria and (linked) management actions are discussed, these crossed with the "bright side" represented by their biotechnological valorization. Moreover, an exercise will be presented considering the potential economic impact of working the "bright side" of cyanobacteria through biotechnology approaches, aiming to allow the deepest understanding of the positive consequences of promoting the ecosystems conservation by their biotechnological valorization. Overall, this review aims to be the trigger for innovative approaches to deal with the cyanobacteria-driven environmental and health issues, interconnecting the fields of water quality, water management, and biotechnology.

2. The nuisance potential of cyanobacterial harmful algal blooms (CyanoHABs)

Cyanobacteria are gram-negative bacteria performing photosynthesis (Domain Bacteria: Phylum Cyanobacteria) that can occur singly or grouped in colonies, often forming blooms. A complex interaction of environmental factors linked to global climate change is contributing to cyanobacterial growth and leading to the outcompeting of eukaryotic algae [5] (Figure 1). Higher optimal temperature ranges for growth [10], buoyancy capacity allowing to benefit (better than non-bouyant microalgae) from vertical stratification caused by the water surface warming [4], higher production and accumulation of photoprotective pigments and antioxidant aminoacids supporting protection against increased UV irradiation[11], and even a better efficiency using increased CO_2 levels in the atmosphere as a source of carbon [5], feature the capacities of cyanobacteria in this context (Figure 1). Even under drought conditions, cyanobacteria can be favoured [12], due to increased water residence [10], as well as with the increase in salinization of freshwaters [4]. Finally, diazotrophic cyanobacteria (e.g. *Anabaena*, and *Cylindrospermopsis*) can fix atmospheric nitrogen in heterocysts to grow successfully under nitrogen scarcity [13].

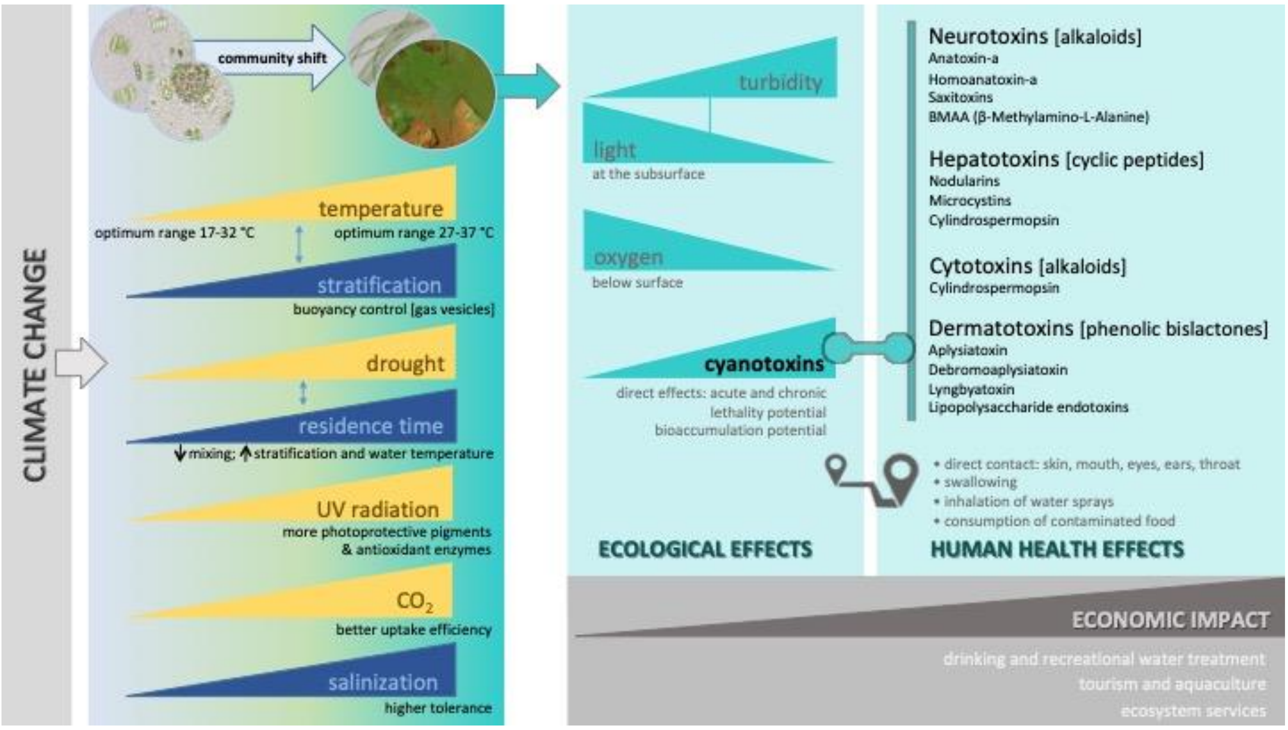

Figure 1. Synthesis on the driving role of climate change towards cyanobacteria dominance over other phytoplankton groups, with emphasis on the consequent noxious effects in ecosystem and human health, as well as negative socio-economic impacts resulting from nuisance management demands.

Harmful algal blooms (HABs) is the generalist name given to algal blooms that affect adversely the environment, plants or animals' health. This is a worldwide nuisance and the greatest public health impacts related to HABs are linked to CyanoHABs, considering drinking water supply and recreational waters [14]. From an ecological perspective, CyanoHABs lead to the reduction of biodiversity and habitat deterioration or even production of toxins [15] as detailed in Figure 1. Consequently, socio-economic impairment occurs, especially regarding affected drinking water reservoirs and recreational waters [16], seed on the treatment demands [17] (Figure 1). While information on HABs prevalence is particularly difficult to find for example regarding Europe, data for USA can be found in scientific and official reports [18]. Costs associated to monitoring programmes can be more easily assessed and are dependent on the monitoring strategy applied and also the area of the lake or reservoir [19]. In large lakes like Taihu (2,537 km^2), and Chaohu (775 km^2), in China, severe events of cyanoHABs imposed the closure of water treatment plants and caused losses of more than RMB 100 million in each lake[19]. The costs associated to additional treatment of water and to the use of additional water sources are also remarkable. These costs can range between $12 million and $56 million for dealing with a problem in a town of 100 000 people, in the USA [20]. More data for USA can be found [e.g. 18], with Bingham et al. [21] estimating $43 million/year losses for recreation and tourism, $18 million for property values, and $4 million for drinking water treatment following cyanotoxin contamination in Ohio. An interesting study by Smith et al. [22], in Canada, demonstrated long-term impacts of blooms. These authors pointed out an equivalent annual cost equal to $272 million (2015 prices) over a period of 30 years, in a business-as-usual scenario. Commercial fisheries affected by problems of eutrophication could generate losses of £ 29-118 000 annually in the United Kingdom [23]. For a more complete compilation of economic data regarding concerning the losses in sectors such human health, commercial fisheries, tourism and recreation, monitoring and management, we refer to the report on the topic to the European Commission by Sanseverino et al. [18]. Still, not many studies focus systematically on the effects of cyanoHABs regarding human health impairment due to the difficulty of establishing consequence links.

2.1. Cyanotoxins

The production of cyanotoxins is associated to specific genes that may or may not be present [24], and even if present, expression occurs depending on environmental conditions [25]. Still, toxic blooms may occur frequently with potential health risks associated to direct contact, bioaccumulation of cyanotoxins through the food chain and/or through the inhalation of water particles due to crop-spray irrigation, and haemodialysis [26]. Cyanotoxins can act as neurotoxins (including neurodegenerative agents), hepatotoxins, cytotoxins, and dermatotoxins (Figure1). Occurrence of cyanotoxins and its association with cyanobacterial *taxa* can be pictured, as previously reviewed [18,26,27], but expectable concentrations are difficult to ascertain due the scarcity of studies addressing the time and space variability of blooms' toxin content, although this is critical information to conduct risk assessment and protective water management [16].

Although sub-lethal doses allow full recovery and no effects of chronic exposure have been observed, neurotoxins can be lethal at high doses by causing asphyxia through paralysis of respiratory muscles [16]. Besides direct action as hepatotoxins, microcystins accumulate in mammals and fish, with consequent tissue and cell damage [27]. Microcystin toxicity is cumulative, and it can be lethal in vertebrates depending on the dose by liver necrosis, within hours to a few days following exposure [28]. Meanwhile, chronic exposure of humans to low microcystin levels in drinking water can promote cancer [15]. In Caruaru (Brazil), numerous deaths occurred by exposure to microcystins in water used for the dialysis [29]. This is the most common toxin found worldwide [30], but cylindrospermopsin is also a concern. It was first discovered after a poisoning incident on Palm Island (Australia) in 1979, when 148 persons were hospitalized with hepatoenteritis due to contamination of a drinking water reservoir [31]. This toxin acts by blocking protein synthesis, potentially causing kidney and liver failure [32]. Besides acutely hepatotoxic, cylindrospermopsin is genotoxic and potentially carcinogenic [33].

While cyanotoxins can entry in the human body *via* direct contact, swallowing or inhalation during recreational or occupational exposure to contaminated water, less common exposure routes are through renal dialysis, irrigation water used in crops and possible uptake into the food chain and dietary supplements [34].

3. Brief notes on the regulatory appraisal of cyanoHABs

The nuisance potential of cyanoHABs was ignored in socio-political arenas worldwide until the 1990s, when evidence of environmental and human health impairment by cyanobacteria and cyanotoxins became exposed. The United States Environmental Protection Agency (USEPA) included freshwater cyanobacteria and their toxins in the first Candidate Contaminant List in 1998, and since then, regulatory agencies worldwide started considering cyanotoxins as potential bioterrorism agents [35]. In the European Union (EU), the main piece of legislation concerning water quality management is the Water Framework Directive (WFD - 2000/60/EC), establishing the concept of ecological water quality, while the specific Bathing Water Directive (BWD - 2006/7/EC) regulates the quality of recreational waters. Phytoplankton is a defined indicator of the ecological quality of water by the WFD; although this includes Cyanobacteria, no guidelines specifically targeting cyanotoxins are given. Regarding the BWD, HAB-causing organisms are neither assessed in practice nor given a relevant role in qualitative classification ruling management actions, although cyanobacteria are specifically mentioned as part of the bathing water profile. Currently, a new Directive is under preparation, which will certainly follow the recommendations by the World Health Organization (WHO) on cyanobacteria. These recommendations include on-site public awareness on the risks in susceptible areas following on dedicated monitoring of cyanobacteria biovolume, chlorophyll-*a*, phycocyanin, water transparency or cyanotoxin concentration [36]. An important example of supportive WHO guidelines are those for safety levels in drinking water and recreational waters that were recently published [26]. This document suggests several guideline values for several cyanotoxins. These guidelines are based on animal studies, except for saxitoxins due to the rapid onset of highly specific symptoms caused by the consumption of contaminated seafood. The provisional lifetime drinking-water guideline values are of 1 $\mu g.L^{-1}$ for microcystin-LR, and 0.7 $\mu g.L^{-1}$ for cylindrospermopsin, while the provisional short-term drinking-water guideline values are of 12 $\mu g.L^{-1}$ for microcystin-LR, 3 $\mu g.L^{-1}$ for cylindrospermopsin, and 30 $\mu g.L^{-1}$ for anatoxin-a and an acute drinking-water guideline value is given also for saxitoxins (3 $\mu g.L^{-1}$). The provisional recreational water guideline values are of 24 $\mu g.L^{-1}$ for microcystin-LR, 6 $\mu g.L^{-1}$ for cylindrospermopsin, and 60 $\mu g.L^{-1}$ for anatoxin-a, while there is a recreational water guideline value of 30 $\mu g.L^{-1}$ for saxitoxins. Besides providing these guidelines, the WHO also suggests an Alert Level Framework, with specific guidelines and steps for both drinking and recreational waters. As each level is met, the WHO suggests specific management measures ranging from the triggering of closer surveillance to the use an alternative water supply or an effective water treatment (in the case of drinking water), as well as bathing restriction (in the case of recreational waters) [26]. In parallel to the WHO protocol, several countries established their guidelines focusing on cyanotoxin concentration reviewed by Chorus et al. [26]. Recently, the European Union released a new Directive on the quality of water for human consumption (EU Directive 2020/2184) that should be transposed to the national legislation of the Member States by 12 January 2023, and microcystin-LR was added to the list of compounds of mandatory monitoring by 12 January 2026 (Part B of Annex 1). The same guideline as used by WHO (1 $\mu g.L^{-1}$ for microcystin-LR) is proposed as a safety benchmark, but this toxin only needs to be measured when there is a risk of blooms occurrence in the water sources.

4. Current management strategies targeting cyanoHABs

Risk management programmes are dependent on the political, social and economic context, as well as on the scientific data available in each region [34]. Despite the inexistence of a "rule of thumb" to deal with cyanobacteria, Alert Level frameworks are a common management instrument, applicable in drinking water facilities [34] and water bodies used recreationally [e.g. 37]. Management can be done taking preventive measures, to reduce nutrient inputs in the water, or control measures, to remove cyanobacteria or their toxins from affected waters.

4.1. Common preventive measures targeting cyanoHABs

The application of preventive measures targeting cyanoHABs requires the identification and limitation of human- or animal-driven contaminants' input into waterbodies. As management at the source level is expensive and time-consuming [38] and given that natural feedback mechanisms in aquatic ecosystems stem from the benthic compartment [25], there is a delay (that can be of several years) between implementation and the significant dropping of nutrient concentrations below levels expected to sustain an algal bloom [25]. Alternative prevention measures have been deemed effective in the short-term, which can be of physical, chemical or biological nature (Table 1) as follows.

The disruption of natural water stratification is meant to favour the access to light of non-buoyant green algae and diatoms [39]. Bubble plume aerators and mechanical mixers are the most common equipment for artificial de-stratification, its efficiency being dependent on the interplay of several operational conditions [39,40]. Such systems were already used successfully to depress cyanobacteria, favouring diatoms and chlorophytes in the Bleiloch Reservoir, Germany [41], but validation is still scarce to assume their broad efficiency [42] and evidence actually exists that the technique can promote rather than control some cyanobacteria species [43]. Water-level fluctuations can be applied to disrupt water stratification every few days for lake-restoration, by controlling the magnitude and timing of the discharge. However, they were not used so far to mitigate cyanobacterial blooms [44], being the removal of sediments to limit sediment release of nutrients to the water column very labour intensive, typically with short-term implications in water quality and questionable ecosystem quality improvement [45]. Hypolimnetic oxygenation decreases the release of nutrients from the sediments without disrupting the stratification of the water body, but requires deep understanding of hydro- and nutrient dynamics [40]. Phosphorus (phosphate) precipitation [46–48] followed with treating the sediment by capping with different agents [40] can also apply to chemically prevent cyanoHABs, while inefficacy has been noted in some systems [45].

4.2. Common control measures targeting cyanoHABs

Chemical control approaches can be very efficient in the rapid deterioration of existing cyanoHABs [e.g. 49], most commonly by the application of coagulants and algaecides (Table 1). Coagulants, such as aluminium and ferric salts [50] deposit cyanobacteria cells preventing access to light, the deposits being then removed mechanically. However, they can in parallel disrupt cell walls and membranes, with the consequent release of cyanotoxins, and residues can easily exceed water quality standards.

New coagulants are being developed with better performance regarding cell lysis [51] and improved

Table 1: Non-exhaustive compilation of current and proposed methods to prevent and control cyanoHABs. Physical (Phys.), chemical (Chem.) and biological (Biol.) methods are covered and examples of entailed agents are given. The highlighted operational constraints (or requirements) to successfully apply each method, as well as advantageous or disadvantageous aspects (relative among different alternatives within each type of method), were interpreted from the literature cited through this work and are more limited for strategies that are still at very early development stages.

	Technique	**Agents/Equipment**	**Operational constraints**	**Advantages**	**Disadvantages**
			Prevention		
Phys.	Destratification	Bubble plume aerators Mechanical mixers	Depth; air flow rates or intensity/duration of treatment; degree of stratification	Do not use noxious chemicals	Results are dependent on the structure of the phytoplankton and even the cyanobacteria community
	Water level fluctuations	Outlets	Manipulation of high volumes of water	Successfully applied for lake restoration. Relevant nutrient decrease by dilution	Affects the whole hydrodynamics, and potentially the non-target biota. Not tested specifically for cyanobacteria.
	Sediment removal	Draglines; dry mechanical removal; hydraulic dredging	Very labour intensive and expensive	Limits sediment sourcing of nutrients	Lack of specificity; may severely affect the benthos
Chem.	Hypolimnetic oxygenation	Airlift pumps Side stream oxygenation Direct oxygen injection	Expensive. Deep knowledge of hydrodynamics, nutrient release and external loads	Improves quality of cool habitats; limits sediment sourcing of nutrients and noxious compounds	Maintenance of thermal stratification, potentially benefiting cyanobacteria.
	P precipitation coupled with sediment capping	Lime, $CaCO_3$, Ca_2O_4Si, $CaCl_2$ Insoluble iron compounds, zeolites, bauxite, clay, calcite, Phoslock^{TM}	Expensive Requires repeated treatment	Effectively reduces phosphate levels and prevents (re)mobilization to the water column	Potentially with severe effects on the benthic biota
			Control		
Chem.	Traditional coagulants	Aluminium salts Ferric salts	Expensive by the need of multiple treatment and removal of deposited residues.	Fast action and effectiveness	Inherent cell lysis. Low specificity - impairs non-target species. Polluting residues. Ti xerogels are more expensive and not comprehensively known yet
	Innovative coagulants	Titanium xerogel		Improved efficacy and easier removal; targets also cyanotoxins	
		Clays, Chitosan, Tannins	Expensive by the need of multiple treatment	Lower environmental toxicity and better biodegradability	Relatively new, thus with inconsistent evidence on efficiency
	Algaecides	Metallic salts (e.g. $CuSO_4$) Photosensitizers (e.g. H_2O_2, phtalocyanines, TiO_2) Triazine herbicides	Used in early bloom stages. Expensive due to the need of multiple treatment. Post-treatment isolation required	Fast action and effectiveness	Possible cell lysis. Low specificity - impairs non-target species.
	Biological chemicals	Polyphenols, nonanoic acids Sanguinarine	Dosing as plant extracts or as purified biochemicals	Less toxic in general and more easily biodegradable	Less efficient. Their modes of action are still not fully known.
Biol.	Biomanipulation	Increase grazing by decreasing zooplanktivory	Depends on the prevalence of edible cyanobacteria species	Environmentally friendlier	Highly complex and with inconsistent efficiency evidence
		Macrophytes	Inefficient in eutrophic waters		
	Biological control	Cyanophages Fungi (parasitism)	--	High cyanobacteria specificity	Easy development of resistances (cyanophages and fungi) or are unspecific.

	agents	Bacteria (Extracellular metabolites) Protozoans (predation)		--	Difficult to isolate and culture at large scale
Phys.	Biomass removal	Oil-spill skimmers Pumps	Application at late bloom stages (scum). Expensive.	Does not use noxious chemicals	Requires further investment to treat removed biomass.

environmental safety [52,53], although with some recognised constraints. Synthetic algaecides [38,49,54] are applied to cause cell lysis, being mostly used in early stages of a bloom to minimize toxin release [40]. Their high and unspecific toxicity ranges are a problem [38], and alternative promising bio-based substances [55] have been studied. Although bearing lower toxicity to other phytoplankton groups and higher biodegradability, these substances are typically less effective and have higher production costs.

Biological strategies emerged as alternatives to chemical dosage (Table 1). These are based on biomanipulation techniques over the trophic structure of the system. Top-down strategies (e.g. favouring grazing by reducing zooplanktivory) fail to control blooms of inedible species (e.g. filamentous morphotypes) [56]. Bottom-up control can be achieved e.g. by manipulating macrophyte density to promote competitive nutrient removal from water [57] and to favour the release of allelopathic compounds to cyanobacteria [58]. Other organisms like viruses (cyanophages), bacteria (through photosynthetic inhibition promoted by extracellular lytic substances [61]), parasitic fungi and predatory protozoa, have been equated [57], but also bearing significant shortcomings [61,62]. Cyanophages are extremely difficult to isolate and/or culture, and cyanobacteria can easily develop resistance on the other hand [61] while there is generally a high specificity of the cyanophage to particular cyanobacterial strains [63]. Parasitism of cyanobacteria by fungi is also possible, but control strategies based on such a biotic interaction are very difficult to upscale [62]. Also, although there are reports on predation over cyanobacteria by several protozoans [64], the suitability of such a strategy can be questioned considering that many cyanobacterial species form colonies preventing that protozoans graze them [57].

Oil-spill skimmers and pumps were successfully applied for the physical control of cyanoHABs (Table 1), especially at scum stages, combined or not with coagulation [65,66]. A successful case where an oil-spill skimmer was used following coagulation with polyaluminium chloride was reported by Atkins et al. [65], regarding a *Microcystis aeruginosa* bloom in the Swan River, Australia. A different system for physical control is the use of pumps to remove dense cyanobacterial blooms.

This system was used in 2007 in Taihu Lake, China, in a bloom that left more than 2 million people with no potable water [66]. However, examples of this kind of physical removal as a cyanoHABs control strategy are very scarce;. Although physical removal may overcome the problem of cell lysis, this strategy does not seem to collect the preference of managing entities, possibly because of added costs of operation, i.e. those related to biomass removal and transport for landfilling or wastewater treatment plants [65]. Given the advantages of physical removal over other control treatments, which are inefficient regarding contamination with cyanotoxins and/or burden ecosystems with hazardous chemicals, the search for solutions to improve the economic sustainability of physical removal is a worthwhile research arena, potentially with important impacts on the successful management of cyanoHABs under a circular economy rationale. In practice, the economic income driven by the valorization of cyanobacteria biomass should easily cover the costs of physical control methods relying on the removal of cyanoHABs and eliminating or greatly reducing waste generation.

5. Cyanobacterial biomass valorization

Climate change will increase the pre-existent risks and create new risks in several environmental arenas, including those imposed by cyanoHABs. Thus, the adaptation or development of solutions contributing to reduce the risk vulnerability and exposure is needed [2]. The relevant drawbacks to efficiently control cyanoHABs make it unable to guaranty the nullification of the threat posed by cyanotoxins or withstand economic sustainability levels. However, the “dark side” of cyanobacteria can be compensated by exploring their “bright side”, integrating cyanoHABs management by physical removal with their biotechnological valorization. Firstly microalgae and, recently, cyanobacteria have been considered amongst the most promising feedstock for biofuels and biochemicals [67]. However, only 10% of the species were partially or totally characterized in this context and only a few are industrially cultured [68], suggesting high losses regarding the exploitation of their biotechnological potential. Besides, cyanobacteria are biochemically rich in antibacterial, antifungal, antiviral, and antitumor compounds, some like polyunsaturated fatty acids and phycobiliproteins already displaying high commercial value [8,9].

5.1. High market value compounds produced by cyanobacteria

Elucidating on the broadness of application possibilities, Burja et al. [69] analysed 424 marine cyanobacteria compounds and found that around 40% were lipopeptides, including compounds with cytotoxic, antitumor, antiviral, antibiotic, antimalarial, and antimycotic activity, as well as multi-drug resistance reversers, antifeedants, herbicides and immunosuppressive agents. Figure 2 synthetises the most valuable cyanobacteria products, along with their bioactivity and application ranges. Carotenoids are light harvesting pigments that are also protective for excessive solar radiation [70]. These are powerful antioxidants and can be used as colorants, applied as food additives, in the cosmetics industry and in aquaculture [71,72]. Phycobiliproteins are water-soluble fluorescent pigment-protein complexes acting as secondary light-harvesting components in the photosynthesis [6]. The primary application of these molecules is as natural dyes, although with potential for the pharmaceutical sector [73] considering their bioactivity [8]. Chlorophylls, the primary photosynthetic pigments, have been used as food colorants [74] and more recently argued as cancer preventive [75]. Although their potential, pigments exploitation is still in its infancy due to low productivities and high recovery costs [70].

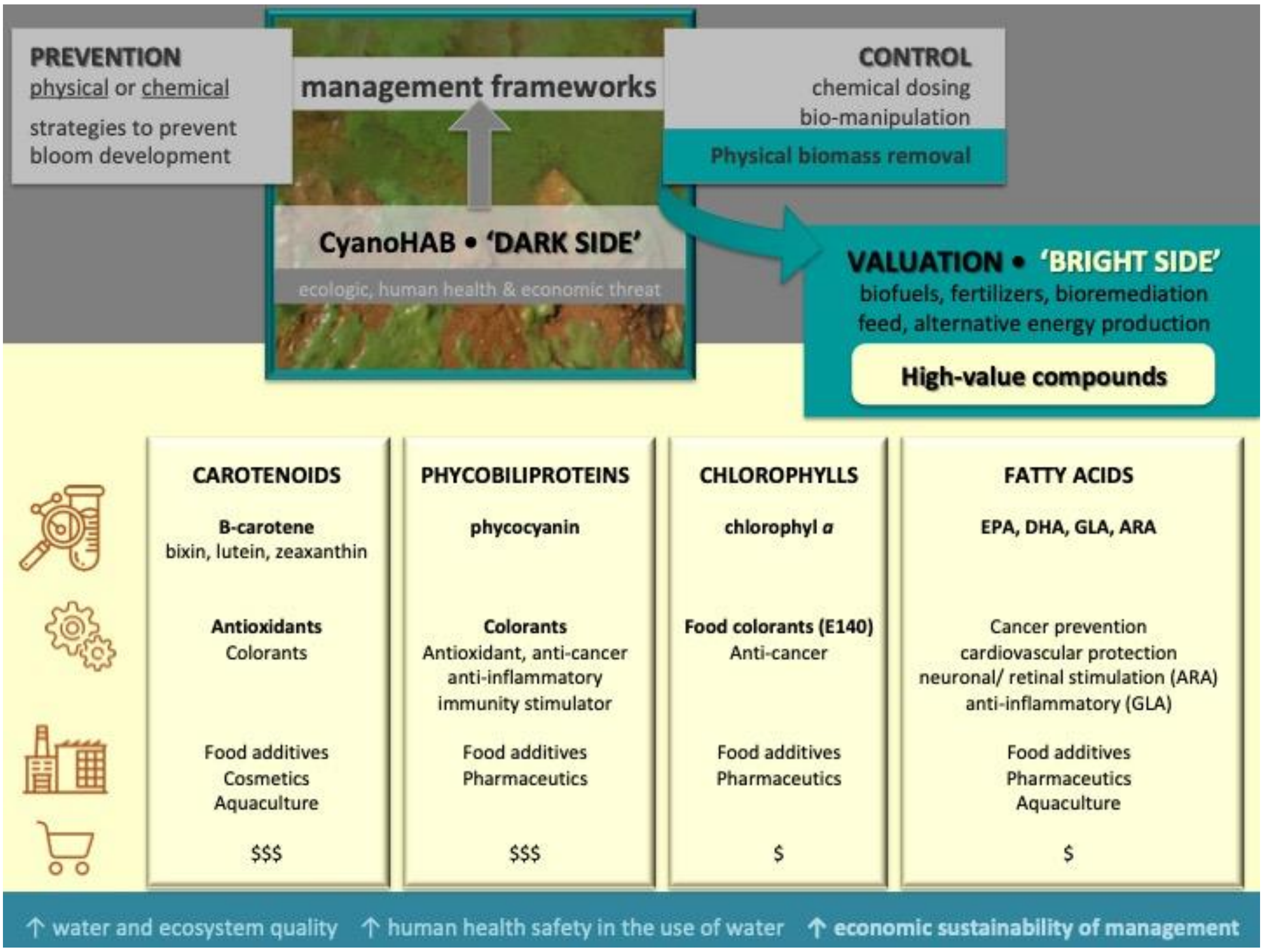

Figure 2. Infographic overview of the proposed strategy for an improvement of management frameworks targeting cyanoHABs, which are growing driven by climate change. The central pillar of the proposal is the valorization of cyanobacteria biomass following control by physical removal, especially regarding the efficient recovery of compounds, along with their bioactivity, application sector and market value being indicated. High-value compounds that were not yet recognised for market size/value estimation were not included and are rather discussed in the text. Icons were made by Eucalyp, Freepik or Kiranshastry from www.flaticon.com (accessed April 2020).

Still, pigments are the cyanobacteria products with the highest valorization potential, particularly carotenoids and phycobiliproteins, the market value depending on the location of production, the current marketing situations, and the product purity [70]. According to authoritative platforms (https://www.gminsights.com, assessed January 2021, for market size and projections), the carotenoids market size reached more than USD 200 million in 2015 and is likely to exceed USD 300 million by 2024, with lutein reaching USD 40 million in 2015. Lutein can be sold in capsules of 18 mg (€ 27.78 for 30 capsules), to improve eye function and prevent macular degeneration (www.nutribio.pt assessed at 24 March 2021). Phycocyanin worth over USD 18.5 million in 2018 and the industry expects a consumption higher than 200 tons by 2025. One of the products in the market is Super Bluecell, from Vegafarma (€ 51.25 for 30 capsules). These capsules use phycocyanin from *Arthrospira* sp. (previously classified as *Spirulina* sp.) and are claimed to be relevant to prevent diseases and aging, protect the organism and prevent secondary effects of chemotherapy or radiotherapy (www.nutribio.pt assessed at 24 March 2021). There is no specific information regarding chlorophylls, but the natural food colorants market, where these pigments are dominant, is projected to surpass USD 4.7 billion by 2024. The majority of chlorophyll that is sold in the market is presented as a liquid and it is extracted from plants like alfafa. Liquid chlorophyll can be dissolved in any drink, arguably to improve health by helping regenerating blood quality and detoxifying. It can be sold by € 15.95 for 100 ml (www.amazon.de assessed at 24 March 2021). The market for lipids (e.g. Omega 3) that can be sourced by cyanobacteria is smaller but still exceeding USD 2.3 billion in 2019 and estimated to reach USD 3.8 billion in 2026. An example of a product that is already in the market is ALGAE DHA, by Nordic Naturals. This Omega-3 supplement is argued to supports optimal brain, eye, and nervous system function, as extracted from the microalgae *Schizochytrium* sp. - 60 capsules of 500 mg can cost € 25.75 (www.iherb.com assessed at 24 March 2021). This valorization route for cyanobacteria lipids is nevertheless apart from their appreciation to produce 3rd and 4th generation biofuel [68]. In fact, many cyanobacteria polyunsaturated fatty acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), γ-Linolenic acid (GLA) and arachidonic acid (ARA), all having key roles in human metabolic and physiological processes [76–79], can be used for nutraceutical, pharmaceutical and therapeutic applications (Figure 2; [80–82]), such applications representing a much higher economic income than biofuels [70].

Besides the above applications entailing an already established market, cyanobacteria are also valuable for their polysaccharides (extracellular polymeric substances). These compounds can be used as bioflocculants by the water treatment industry [83]; emulsifiers, stabilizers or thickening agents [84] in the food industry; bioactive substances, e.g. sulphated polysaccharides that can inhibit tumour invasion and metastasis [85]; and bioremediation agents due to their metal binding capacity [86]. Cyanobacteria can also produce poly(hydroxyalkanoates) [87], defined as potential substitutes to conventional plastic [88]. These polyesters have similar applications as polypropylene, but are biodegradable, and related manipulation technologies are already widespread at the industrial scale [89]. These compounds were already described in several cyanobacterial species [87], and since they have smaller nutritional needs when compared to heterotrophic bacteria, their use as industrial PHA producers is more appealing considering that the costs associated to the microbiological production of the latter were a constraint [89]. Specific high-value metabolites have been extracted from cyanobacteria, with the most remarkable cases being (i) cyanovirin-N (CV-N), a protein produced by *Nostoc ellipsosporum* able to inactivate some primary strains of HIV-1; (ii) borophycin produced by *Nostoc* species with cytotoxicity against human epidermoid carcinoma and human colorectal adenocarcinoma cells; and (iii) cryptophycin, also isolated from *Nostoc* strains with fungicide activity and cytotoxic action against human tumour cells [69]. The production of secondary metabolites is generally induced by stress conditions [90], which can meet environmental challenges such as increased salinity, drought or temperature consequent to global climate change trends.

Apart from extracted metabolites, whole cyanobacteria have been proven to be competent in many other fields. They are apparently good bioremediation agents for metal contamination [91], can be used successfully as fertilizers [92], as feed supplements in aquaculture or as hosts for the synthesis of nanoparticles [e.g. 93]. The third generation biofuels such as biohydrogen, biomethane, bioethanol and biodiesel, can be produced using cyanobacteria [68] and their potential for integration in microbial fuel cells for electricity production has been investigated [94]. Although the actual value of these applications is still largely unquantified, the markets involved render a significant economic potential, more even considering that cyanobacteria do not compete with plants for (scarce) fertile soil.

6. Improving cyanobacteria exploitation: economic and sustainability aspects

The market of valuable products retrieved from cyanobacteria is increasing. This is being reflected in the increased number of industries producing and exploring this biomass, as well as in research efforts (indicatively, a search in ISI® WoS by March 2021 using the terms 'cyanobacteria' and 'biotechnology' retrieved a total of 387 hits, 16% published from 2020 onwards for the Core Collection database, or a total of 2626 hits, 5% published from 2020 onwards when retrieving from all databases) and in the high pace of discovery of novel applications, as highlighted previously. Some examples of companies spread around the world working in this market are Transalgae (Israel), AlgaEnergy (Spain), Algae Systems (USA), Algenol (USA), IHI NeoG Algae (Japan), Pond Tech (Canada), Necton (Portugal), Cyanotech (Hawaii), Taiwan Chlorella (China). Multinational companies are also investing in this context, some heavily such as ExxonMobil in the field of fuel production (see the onset of collaborative efforts with research institutes in [e.g. 95] and in the company's dedicated website).

The use of natural biomass, especially nuisance biomass, to retrieve benefits while concomitantly offsetting environmental problems, is a smart innovation concept that allows building a win-win relationship between environmental and economic sustainability. These 'mutualistic' relationships are a key solution especially in a future where global climate change is evident and nuisances such as cyanobacteria are expected to grow worldwide. Realistically, regardless how exciting are academic exercises exploring the potential of natural products or supporting the need of improving environmental management to protect ecosystems and ensure the quality of natural resources, economic variables will always be of paramount importance in defining the success of any newly proposed solution. In this field, strategies that allow an integration of cyanobacteria biomass valorization in nuisance management frameworks towards environmental restoration and human-health protection (see Figure 2 for an overview of this suggested approach) are definitively worth of further attention.

As previously detailed, control strategies based on the removal of cyanoHABs biomass are currently not particularly suitable, and we believe that this can be because they are not sufficiently explored. Our reasoning is essentially based on the valorization of removed biomass to cover the costs of operation, and depending on the design of valorization routes, the income may additionally compensate to a certain extent for the overall damage caused by the cyanoHABs (i.e. economic costs of ecological and public health threats). This perspective is illustrated in Figure 2 as the change in focusing the “bright side” of cyanobacteria instead of their “dark side”. Such an add-on to nuisance management frameworks was already suggested e.g. for the invasive macrofouling bivalve *Corbicula fluminea* by Rosa et al. [96] and Domingues et al. [97]. An important remark stressed in these works where profitable features of invasive species are exposed regards the need to absolutely prevent further dispersion, while applying the proposed strategies to avoid the scale-up of noxious environmental and economic impacts. This principle should be extended to the valorization of cyanoHABs. Our proposal stems from the idea that cyanoHABs have potential to be valued, but it is strict in the sense that this should be done when blooms cannot be prevented and are already established. Moreover, its most prominent perspective is the promotion of the economic sustainability of blooms control and the concomitant stimulation of water quality improvement, which immediately down tones the relevance of business-based approaches requiring, for example, especial and temporal stability of the biomass source; on the other hand, the framework is not preventive of an association between environmental management entities interested mostly in the control of the nuisance and business-driven sectors, depending on local regulation on the exploitation of natural resources, the availability of the biomass and the relationships allowed between the sectors.

Ideally, a process where multiple valuable products can be retrieved from the same biomass, as suitable in biorefinery processes, should be considered since this allows to magnify the incomes

[68,98]. As suggested by Chisti [98] for microalgae, cyanobacteria biomass can be used to concomitantly produce biodiesel, animal feed and biogas, the costs of feedstock production being reduced by natural availability, which contributes further to the efficiency of the valorization routes. Less positive aspects of the valorization of cyanoHABs within a biorefinery framework are two-fold. On the one hand, blooms are seasonal in temperate areas (note that the magnitude of this problem severely decreases in tropical and sub-tropical areas), but climate change (mostly rising temperatures and altered hydrologic patterns) is changing blooms occurrence [3–5], which translates in the extension of the periods through each year when this biomass is available. Indeed, related changes in the phytoplankton community composition have been observed [5], as well as the geographical expansion of cyanobacteria [3]. Still, although cheap, cyanoHABs biomass is spatially and temporally variable, so as it is its quality and, consequently, the yields regarding different valuable products. A way to overcome both shortcomings is the establishment of culturing facilities that can be activated when natural feedstocks cannot be collected. The culturing of cyanobacteria/microalgae at the industrial scale is viable (see the example of Solazyme, Inc., San Francisco), and has potential for improvement, for example, through genetic engineering of the cultured strains [67] or by the implementation of integrated culturing systems that allow reducing the costs associated to the creation of culturing facilities. These systems can use wastewater treatment facilities, rich in nutrients, to produce algal biomass while retrieve part of the organic load from waters. Systems like this, transform organic waste in algal biomass, closing the cycle - see e.g. Rose et al. [99] or Nagy et al. [100] for the context, structure and possibilities of integrated algal ponding systems. These systems not only contribute generally to a sustainable development but also to improve the sanitation conditions, allowing waste recovery, recycle and reuse, enabling societies to live from its nature income rather than consuming its capital [99].

6.1. Rendering the concept concrete from collection to exploitation – a reasoned exercise

Realistically, economic dynamics cannot completely dissociate from environmental protection, and this in an important trigger to develop sustainable solutions for environmental problems. This concept of blooms valorisation does not entail a traditional or even a straightforward approach, but it has the potential to effectively build a bridge to bring economical profit to the side of ecosystems conservation, hence favouring environmental protection and restoration. In the present section, we propose a structured theoretical framework to build this bridge, rendering the overall approach economically and environmentally productive, and thus sustainable. This framework is proposed and inspired by the circular economy philosophy, being composed of four sequential, internally flexible steps (Figure 3): step 1 would comprise collection, transport and eventual storage of the blooms intended for valorization; step 2 would include biomass characterization; step 3 would comprise the selling of the biomass to extraction facilities; and step 4 would entail the application of the potential profit or Return (R) retrieved from the selling of the biomass.

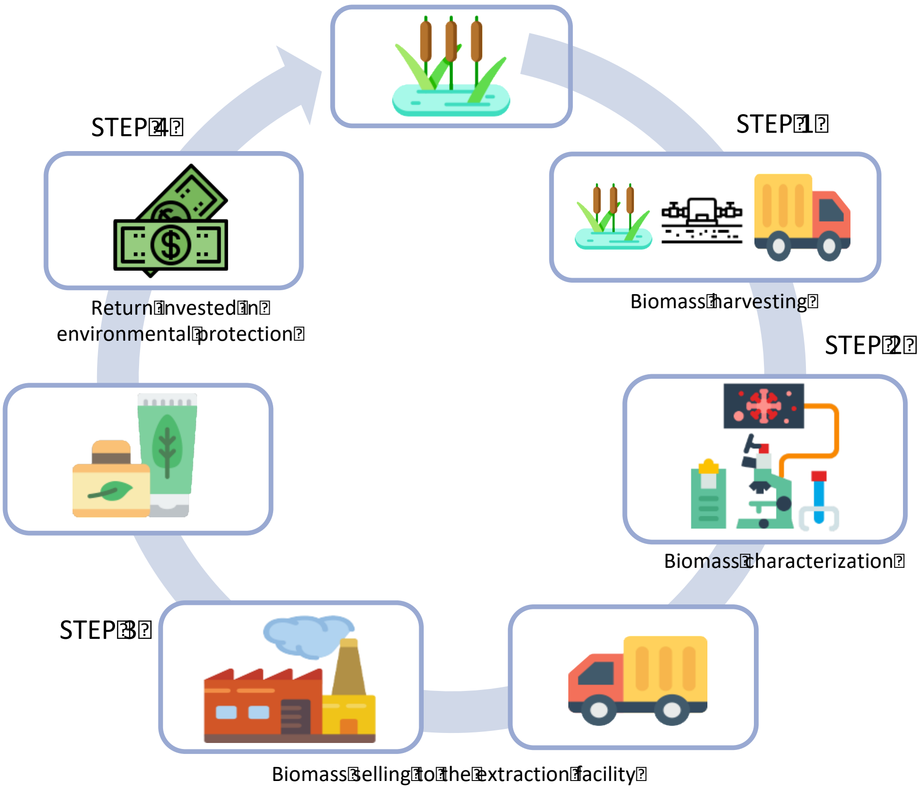

Figure 3. Schematic illustration of the proposed concept of integrating the biotechnological exploitation ('bright side') of cyanobacteria natural blooms to improve the efficiency of

management frameworks, as inspired by the circular economy philosophy. The scheme includes four sequential, internally flexible steps. Icons from www.flaticon.com (accessed January 2021).

The sequence towards the sustainable valorization of cyanobacterial blooms obviously starts with the identification of an affected waterbody. Cyanobacterial biomass can be extracted (i.e. removed) by suction of water with circulator pumps coupled with sieves so that retained cyanobacteria can be easily collected into a container in a truck (Step 1). Systems for extraction of blooms and concentration of the biomass are increasingly available, as illustrated by e.g. patent CN101602533B or the work of companies like AECOM [101]. The cells retained in the sieves cab be scraped into an appropriate container for transport. The type of pump or oil-spill skimmer used for collecting the cyanobacteria should be carefully optimised as it constitutes an important part, if not the most significant part of the costs associated to the proposed valorization strategy. Furthermore, depending on the stage of the bloom being collected, sieving optimisation can be required. At an advanced stage (scum or nearly-scum phase), cyanobacteria accumulate mostly in the surface, and pumping only from surface water layers prevents the capture of non-target organisms. When this is not the case, the sieving system requires optimisation, for example by assembling sequences of sieves of decreasing mesh size. While cyanobacteria should be retained by the finer sieve(s), the accumulated biomass being easily scrapped into the transport container, non-target organisms retained in larger upstream sieves can be easily returned to the ecosystem by back flushing. The cyanobacteria biomass is then transported into a processing facility where it is first stored. The storage process (e.g. refrigerated chamber) prevents the degradation of the biomass and the production of bad odours, until further biomass characterization, *i.e.* toxin analysis (Step 2).

Step 2 is a critical step because the presence of toxins will determine the fate of the biomass. Safety measures are important when handling potentially toxic cyanobacteria. Protective equipment range from standard laboratory coats, gloves and safety glasses to breathing masks if there is a risk of inhalation [26], although this equipment does not largely differs from basic safety equipment recommended for handling natural samples from any aquatic ecosystem that is not comprehensively known by operators. Naturally, the products extracted from a natural bloom may not be the same as the ones extracted from pure cultures of cyanobacteria. Compounds with high purity requirements such as those intended for food or pharmaceutical applications cannot be the primary targets of blooms collected *in natura*. However, there are suitable and economically attractive applications for such a raw biomass, including biofuels, fertilizers, but also the extraction of less purity-demanding compounds and even toxins. If toxins are present, the biomass should be treated carefully, and its destination could be the extraction of the toxins itself or the production of biofuels. If the biomass is potentially toxin-free, other uses can be equated (see section 5 for an overview) complying with the applicable regulation within each envisaged market. Therefore, toxin screening is critical, and can be made through several approaches. Molecular approaches based on the identification of genes encoding for cyanobacterial toxins can play an important role as they are simple, rapid, cost effective, sensitive and specific, allowing the simultaneous analysis of several target gene products [102]. The presence of these genes can be easily assessed by PCR (Polymerase Chain Reaction) using primers targeting regions of the operons involved in the synthesis of microcystins [103], cylindrospermopsin [104], saxitoxins [105], anatoxins [106], and nodularins [107]. However, the presence of target genes does not necessarily mean that they are transcribed and that transcripts are actually expressed for the actual production of the toxins by the cells. Still, this screening stage is relevant: if samples do not bear genes responsible for toxins production, then there is no need to invest in further stages (some more expensive) to confirm the presence of toxins in the sample; depending on the target genes present, then a selection of downstream methods for toxin quantification can be made, with the logical cost efficiency gains. In this context, methods like the Enzyme Linked Immune Substrate Assays (ELISA) provide the concentration of a particular toxin in a tested sample. This is the most common biochemical technique for cyanotoxins screening and it is based on the coating of well plates or test tubes with toxin antibodies [40]. It is a relatively inexpensive, simple, fast, sensitive, specific and easy technique [34]. However, it also presents some limitations, as it does not distinguish toxin variants. Although this can be a limitation in some studies, in stage 2 of the proposed framework, a precise analysis of the toxins variants is generally unnecessary as the purpose of this biomass characterization stage is to support the decision on directing the raw biomass for different extraction companies, who then must design further assessment schemes depending on the application intended for the extracted product and also the extraction and purification processes that will be adopted. Other options for the general characterization of the raw biomass may include analytical methods such as High Performance Liquid Chromatography (HPLC) [e.g. 108], Mass Spectrometry (MS) [e.g. 109], or even, although less used, the Nuclear Magnetic Resonance (NMR) [110]. A overview on the methods and recommendations can be found in the latest WHO guidelines [26], in Chapter 14. The safety limits that should withstand for cyanotoxins in the collected biomass can be generally interpreted from those suggested by WHO for recreational water. Still, the requirements of each application should tune the definition of each specific set of limits, accounting to the planned processing stages since some of the steps involved (e.g. heat treatment, pH, use of specific solvents) can possibly inactivate some cyanotoxins or even allow successful separation of the toxins while extracting the product of interest.

Once the biomass is characterized, the basic conditions are set for selling it to the most suitable company. A myriad of companies is available (see section 6) and, according to their industrial processes, biomass will be processed and transformed to different products that can then enter in the value chain (Step 3). Finally, considering environmental ethic principles ruling worldwide, it is straightforward that a step 4 is in place to ensure compensatory measures. The most logical proposal is that the profit/return retrieved from the valorization of natural blooms (generally regarding the selling of the biomass to exploitation companies; see below) is re-invested in environmental protection in general or in control by physical removal of blooms (eventually synchronized with step 1 of the valorization framework) more specifically, picturing the closing of the cycle, as illustrated in Figure 3.

In terms of framework management, the most efficient system would be to address the collection, transportation, storage and toxin analysis to local/regional entities with responsibilities towards environmental management of waterbodies (e.g. municipalities, public/non-profit water treatment companies/organizations, environmental management entities). This straightforwardly allows the use of the profit/return retrieved from biomass selling to exploitation companies either to compensate for the expenses in the physical removal of the blooms or to broadly apply in ecosystem protection and restoration measures. Assuming that such a straightforward approach is not universally realistic, a system of fares can be established in benefit of standard management entities in each country/region, so that private companies profiting from each of the steps of the framework can support environmental protection following the users-pay principle [111]. A final note is worth making on the need to tightly regulate the actions under the scope of valorization frameworks. In the case of our bloom valorization framework, regulation is critical to assure that the bloom is not assumed as a normal profitable asset, because this is not primarily a business model. Instead, the idea is restricted to the stimulation for an effective control of existing blooms when preventive management was not effective. Although we obviously do not have real data to evaluate the economic viability of this approach, its preliminary assessment before any future implementation is critical (section 6.1.1). This preliminary assessment was made without a specific assignment of the return to each stakeholder involved. This was done for simplification at such an early stage of the idea, but also because the structure of the consortia involved in its future implementation are very much dependent on the scale (national, regional or local), the overall governance structure regarding environmental management of natural resources at each country or region, and the managerial resources available, including personnel, infrastructure and budget. For example, depending on the context, dedicated financial support from governmental sources can assist the control by removal of cyanobacteria blooms in emergent situations (as reported e.g. for Lake Taihu, China [66]. In such a case, this funding adds to the return that can be obtained through the valorization, reinforcing the stimulation towards effective environmental management of blooms in particular and/or environmental protection and restoration actions in general since the scope for budget enlargement necessarily increases. It is worth mentioning in addition the currently unaccounted benefits that can be gained regarding local and regional development through smart specialization as allowed by the biotechnology-based valorization approach suggested.

6.1.1. Economic viability of the proposed concept

The potential market of removing cyanobacteria from contaminated waters and commercially exploring the biomass was economically analysed, based on the creation different scenarios. Briefly, when an economic analysis is under development, three areas need to be fulfilled to complete the evaluation. It is required (i) to set up a target output or production scenarios, then (ii) to determine the sequence of unit operations/steps and their respective parameters and conditions, and finally, (iii) to collect the economic datasets to populate the model. Following this rationale, some attempts have been approached by some of us aiming to develop the best approach to evaluate the use of biotechnological processes on the valorization of different biomass sources [112,113]. Specifically, in the recent work by Martins and collaborators [112], the valorization of a marine raw material (red macroalga, *Gracilaria gracilis*) through the exploitation of the economic and commercial potential of extracted and purified phycobiliproteins was successfully addressed. Using a similar approach, the parameters of potential profit or Return (R) were defined and modelled herein. Briefly, this model/equation generates an R value that can be a positive or negative number, which represents the potential profit that can be generated from a product by considering its production, potential price, and production costs. The simplified version for the Return equation (Eq. 1) used herein relates the Return to the concentration of cyanobacteria in the contaminated water (mass *per* unit of volume) (C_{cyano}), the potential selling price for this biomass (price *per* unit of mass) ($\$_{cyano}$), the associated administrative ($\$_{admin}$) and process ($\$_{proc}$) costs for harvesting the cyanobacteria (cost *per* unit of volume).

$$R = \left(C_{Cyano} \times \$_{Cyano} - \$_{Admin}\right) - \$_{Proc} \qquad \textbf{Eq.1}$$

As previously mentioned, different scenarios were created to provide a more complete evaluation. For a more comprehensive analysis, a large range of values for each variable was defined. It was decided to test for each of them the range from 0.001, then by increasing by a factor of 2 up to reaching the first value above of 100 (this limit was decided as it is possible to analyse any number of desired scenarios, but a limit must be established). These values were applied for each variable, and the units can be expressed in any currency, mass, and volume unit, as long as they are consistent throughout the equation. In the case of the example presented here the units were either in $g.L^{-1}$, Euro (EU).g^{-1}, or $EU.L^{-1}$. In total, 18 values for 4 variables provided a collection of 104,976 combinations. The complete collection of results for the Return calculation can be found in the Supplementary Material. Within this range of values for R obtained by the 104,976 combinations the potential real (in practice) value can be found.

For this work, it was decided to use the value of EU 103 *per* 21,000 L (EU 0.0086 *per* 1 L) as a benchmark for the costs of harvesting cyanobacterial biomass from a contaminated waterbody (i.e. process costs, $\$_{proc}$). This is a median value charged by some Fire Departments in Portugal to fill private pools, following pumping from a natural reservoir and transporting 21,000 L of water. We argue that the costs of the Step 1, regarding the collection, transport and possibly short-term storage of the biomass (Figure 3) should not be much higher/different that this reference value, considering our reasoning that public services with own resources should be involved at this stage (see section 6.1). Notwithstanding this, most of the scenarios created use a cost higher than this (> EU 0.008 *per* 1 L), which will make this study a more robust tool to predict the potential real-life costs and Return.

Since the Return indicates the potential profit, it should have a positive value. In order to enhance the impact of the potential Return, it was decided to present the results for the worst scenario calculated. This scenario was achieved by using the maximum value (EU $131.072.L^{-1}$) for the variables $\$_{Admin}$ and $\$_{Proc}$. In this scenario, the values for C_{cyano} and $\$_{cyano}$ that provided the least positive R value were selected and presented in Figure 4. To preserve a positive value, the combinations of C_{Cyano} and $\$_{Cyano}$ will need to be maintained at the upper right area of Figure 4. In terms of values, this means that a C_{Cyano} of 131.072 g.L^{-1} can have a $\$_{Cyano}$ of EU 2.048.g^{-1}. The same relationship applies for the opposite, i.e. a value of $\$_{Cyano}$ of EU 132.072.g^{-1} can accommodate a low cyanobacterial concentration in water of 2.048 g.L^{-1}. It is critical to understand that these values can be read as high, but they are under the assumption of the worst scenario for $\$_{Admin}$ and $\$_{Prod}$. If these two variable ($\$_{Admin}$ and $\$_{Prod}$) values decrease, then the curve in Figure 4 will shift towards the lower left, allowing room for different combinations of C_{Cyano} and $\$_{Cyano}$, particularly combinations with less favourable values.

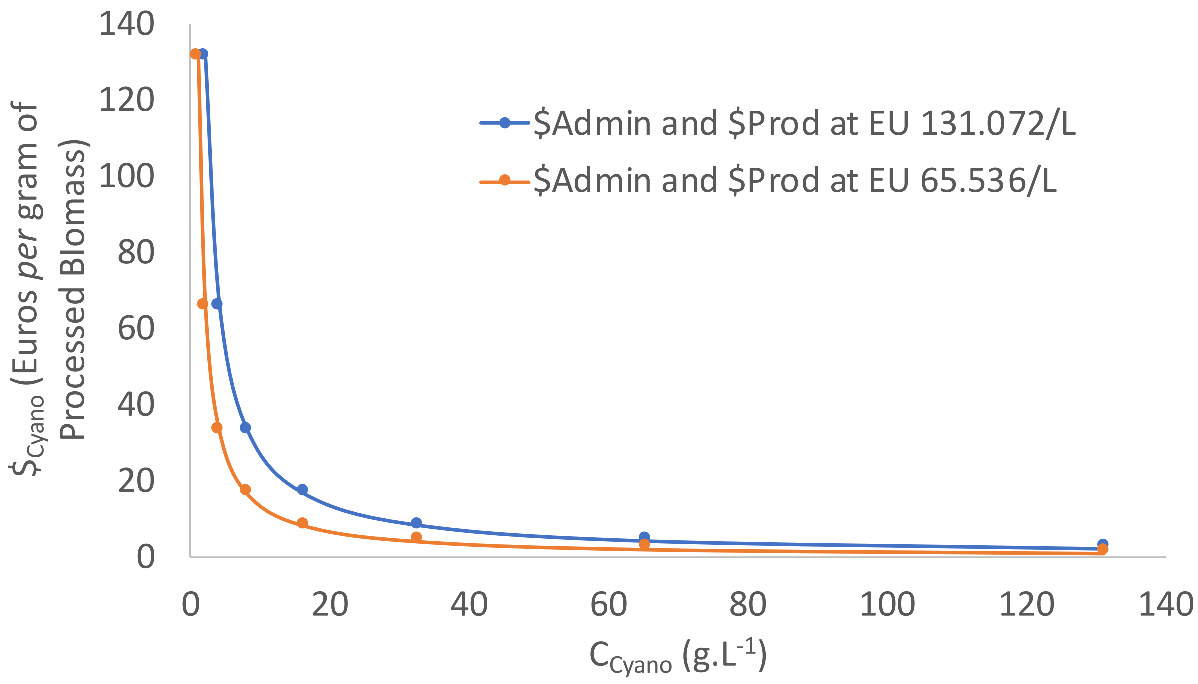

Figure 4. Threshold for a positive Return (R) for a $\$_{Admin}$ and $\$_{Prod}$ at EU 65.536 per liter and EU 131.072 *per* liter. Combinations of $\$_{Cyano}$ and C_{Cyano} for a positive R need to be to the upper right of the curves.

To further emphasize the applicability of the framework proposed herein, the economic analysis exercise can be continued by considering a specific extractable compound of interest. In fact, the biomass collected from natural blooms can be explored for different bioproducts that can be used for different applications in different markets (depending on their toxin content), and this can be incorporated in **Eq. 1** as long as sufficient data are available, which is not so common currently. Still, as an example for which own information is available, we are considering phycocyanin as a model product [114], and the Return analysis was added with two terms (**Eq. 2**) to account (i) for the potential income of selling phycocyanin (C_{Phyco} x $\$_{Phyco}$); and (ii) for the production cost of this pigmented product ($\$_{Phyco\ Proc}$)

$$R = \left(C_{Cyano} \times \$_{Cyano} - \$_{Admin}\right) - \$_{Proc} + \left(C_{Phyco} \times \$_{Phyco} - \$_{Phyco\ Proc}\right) \qquad \textbf{Eq.2}$$

To populate the **Eq. 2** and analyse the impact of including the profit and cost of phycocyanin production, benchmark values were established for the variables in order to decrease the number of calculated critical scenarios. Representing the worst-case scenario regarding the variables in **Eq. 1**, C_{Cyano} and $\$_{Cyano}$ were fixed at the lowest level possible (0.001 grams of biomass *per* liter of water and EU 0.001 *per* gram of biomass, respectively), while $\$_{Admin}$ and $\$_{Proc}$ were fixed at the highest value analysed before (EU 131.072 *per* liter of processed water for both). For the new variables present only in **Eq. 2**, C_{Phyco} was fixed at a reported value of 63 mg of Phycocyanin *per* g of fresh biomass [114], while $\$_{Phyco}$ was allowed to vary within the range comprising food- and analytical-grade prices (EU 0.13 and EU 15 *per* mg of phycocyanin)[114,115]. The last variable is the production cost of obtaining phycocyanin ($\$_{Phyco\ Proc}$). Its value can change dramatically depending on the process used. In this way, and following the literature [112], a convenient range of five values (EU 0.01, EU 0.1, EU 1, EU 10 and EU 100 *per* g of biomass) was tested. The required phycocyanin units conversion from EU *per* gram of biomass to EU *per* litre of processed water was made based on previous studies (100 grams *per* litre [114]). Thus, and to emphasize the application of the proposed framework, the value used here was decreased to 1 gram *per* litre to show results under a restricted situation. Figure 5 shows the two extremes of the scenarios calculated, evidencing R for phycocyanin obtained at food grade or at analytical grade. The R for any other purity grade in between will be located in between. It is worth remarking that the rest of the variables were fixed at the most conservative value (worst-case scenario). In practice, it is expected that these values can be optimised, which will translate into an even higher potential Return.

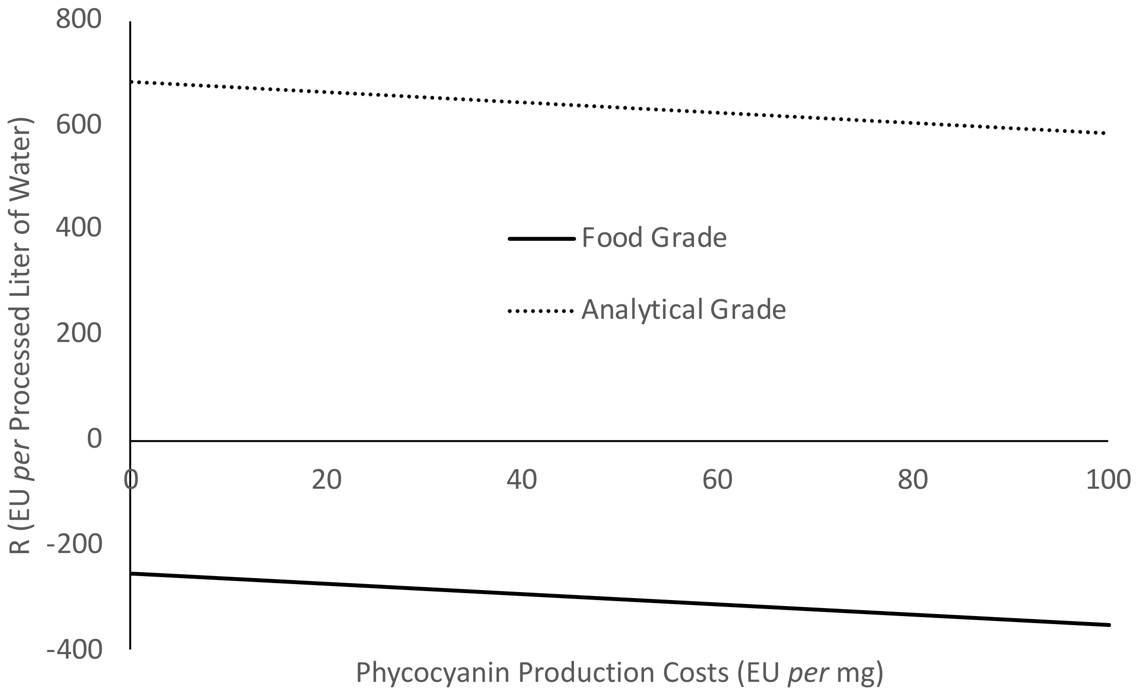

Figure 5. Calculated Return (R) after the inclusion of phycocyanin production. Values for fixed variables are: $\$_{Admin}$ = EU 131.072 *per* L, $\$_{Proc}$ = EU 131.072 *per* L, C_{Cyano} = EU 0.001 g *per* L, $\$_{Cyano}$ = EU 0.001 *per* L, C_{Phyco} = 63 mg *per* g, $\$_{Phyco}$ = EU 0.13 and EU 15 *per* mg (for food and analytical grade, respectively). Results show limits for the potential R that can be obtained depending on the final purity, selling price and concentration of phycocyanin generated.

Conclusion

Climate change is increasing the frequency, duration and intensity of CyanoHABs, imposing growing impairment of the water supply quality, fisheries and recreational resources. Herein, a critical review on the nuisance potential of cyanoHABs ('dark side') and related management practices was followed by the conceptual proposal of integrating their biotechnological exploitation ('bright side') as a new axis to improve the efficiency of management frameworks. The basic protocol within this approach would be to value the cyanoHABs biomass physically removed from affected waterbodies rather than landfilling or directing it to (expensive) treatment, which is a traditional control strategy. Since cyanobacteria are rich in bioactive compounds of high commercial potential, they may be recognised as valuable alternative feedstocks for strategic sectors, under a multi-product scenario. We demonstrate that the economic income of such approach can cover the costs of control actions over existent cyanoHABs, maintaining the direct ecological, economic (in touristic areas), and human health safety benefits, but it can also be profitable. This was evidenced both at the level of the biomass delivery to production units and considering the full exploitation cycle using the valuation of phycocyanin as an example. Although the economic potential and viability of the overall concept explored in this work is already promising, there is further scope for growth if biorefinery frameworks for multi-product valorization from the same biomass are integrated [116]. As a novel proposal for the sustainable control of cyanobacteria blooms, this work is a stepping stone rather than a final draft of a new framework. The present article demonstrates the suitability of the framework, but several aspects certainly need further development stages, both from the operational and the regulatory viewpoints. For example, the design of an efficient pumping system that allow the collection of the cyanobacterial biomass while causing minimum disturbance in the biotic communities of the affected waterbody is a primary aspect deserving attention, although most of the current strategies applied to the control of cyanobacteria blooms also have these kind of drawbacks.

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References

1. IPCC. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (2014).
2. IPCC. *Climate Change 2014 - Impacts, Adaptation, and Vulnerability. Part A: Global and Sectorial Aspects.Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (2014).
3. Paerl, H. W. & Huisman, J. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* **1**, 27–37 (2009).
4. Paerl, H. W. & Paul, V. J. Climate change: Links to global expansion of harmful cyanobacteria. *Water Res.* **46**, 1349–1363 (2012).
5. Visser, P. M. *et al.* How rising CO2 and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae* **54**, 145–159 (2016).
6. Pandey, V. D., Pandey, A. & Sharma, V. Biotechnological applications of cyanobacterial phycobiliproteins. *Int. J. Curr. Microbiol. Appl. Sci.* **2**, 89–97 (2013).
7. Kamal, A. & Ahmad, I. Z. Cyanobacteria “the blue green algae” and its novel applications: A brief review. *Int. J. Innov. Apllied Stud.* **7**, 251–261 (2014).
8. Sekar, S. & Chandramohan, M. Phycobiliproteins as a commodity: Trends in applied research, patents and commercialization. *J. Appl. Phycol.* **20**, 113–136 (2008).
9. Eriksen, N. T. Production of phycocyanin - A pigment with applications in biology, biotechnology, foods and medicine. *Appl. Microbiol. Biotechnol.* **80**, 1–14 (2008).
10. Paerl, H. Mitigating Harmful Cyanobacterial Blooms in a Human- and Climatically-Impacted World.

Life **4**, 988–1012 (2014).

11. Carreto, J. I. & Carignan, M. O. Mycosporine-like amino acids: Relevant secondary metabolites. Chemical and ecological aspects. *Mar. Drugs* **9**, 387–446 (2011).

12. Smith, M. J., Shaw, G. R., Eaglesham, G. K., Ho, L. & Brookes, J. D. Elucidating the factors influencing the biodegradation of cylindrospermopsin in drinking water sources. *Environ. Toxicol.* **23**, 413–421 (2008).

13. Moisander, P. H. *et al.* Facultative diazotrophy increases Cylindrospermopsis raciborskii competitiveness under fluctuating nitrogen availability. *FEMS Microbiol. Ecol.* **79**, 800–811 (2012).

14. Backer, L. C. Cyanobacterial Harmful Algal Blooms (CyanoHABs): Developing a Public Health Response. *Lake Reserv. Manag.* **18**, 20–31 (2002).

15. de Figueiredo, D., Azeiteiro, U. M., Esteves, S., Gonçalves, F. & Pereira, M. Microcystin-producing blooms — a serious global public health issue. *Ecotoxicol. Environ. Saf.* **59**, 151–163 (2004).

16. WHO. Algae and cyanobacteria in fresh water. in *Guidelines for safe recreational water environments* **1**, 136–158 (World Health Organization, 2003).

17. Westrick, J. A., Szlag, D. C., Southwell, B. J. & Sinclair, J. A review of cyanobacteria and cyanotoxins removal / inactivation in drinking water treatment. *Anal. Bioanal. Chem.* **397**, 1705–1714 (2010).

18. Sanseverino, I., Conduto, D., Pozzoli, L., Dobricic, S. & Lettieri, T. *Algal bloom and its economic impact*. *European Commission* (2016). doi:10.2788/660478

19. Hamilton, D. P., Wood, S. A., Dietrich, D. R. & Puddick, J. Costs of harmful blooms of freshwater cyanobacteria. in *Cyanobacteria: An Economic Perspective* (eds. Sharma, N. K., Rai, A. K. & Stal, L. J.) 245–256 (John Wiley & Sons, Ltd, 2013). doi:10.1002/9781118402238.ch15

20. Naidenko, O. V., Cox, C. & Bruzelius, N. Troubled waters: Farm pollution threatens drinking water. Environmental Working Group, Washington DC 20009. *Troubled waters: Farm pollution threatens drinking water* (2012). Available at: https://www.ewg.org/research/troubled-waters. (Accessed: 23rd March 2021)

21. Bingham, M., Sinha, S. K. & Lupi, F. *Economic benefits of reducing harmful algal blooms in Lake Erie*. *Environmental Consulting & Technology, Inc. Report prepared for the International Joint Comission* (2015).

22. Smith, R. B., Bass, B., Sawyer, D., Depew, D. & Watson, S. B. Estimating the economic costs of algal blooms in the Canadian Lake Erie Basin. *Harmful Algae* **87**, 101624 (2019).

23. Pretty, J. N. *et al.* Environmental costs of freshwater eutrophication in England and Wales. *Environ. Sci. Technol.* **37**, 201–208 (2003).

24. Kurmayer, R. & Christiansen, G. The genetic basis of toxin production in Cyanobacteria. *Freshw.*

Rev. **2**, 31–50 (2009).

25. Merel, S. *et al.* State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.* **59**, 303–327 (2013).

26. WHO. *Toxic cyanobacteria in water*. (Taylor & Francis, 2021). doi:https://doi.org/10.1201/9781003081449

27. Metcalf, J. S. & Codd, G. A. Cyanotoxins. in *The Ecology of Cyanobacteria II - Their Diversity in Space and Time* (ed. Whitton, B. A.) 651–675 (2012). doi:10.1007/978-94-007-3855-3

28. NHMRC. *Guidelines for managing Risks in Recreational Water.* (Australian Government, National Health and Medical Research Council, 2008).

29. Pouria, S. *et al.* Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. *Lancet* **352**, 21–26 (1998).

30. Srivastava, A., Singh, S., Ahn, C. Y., Oh, H. M. & Asthana, R. K. Monitoring approaches for a toxic cyanobacterial bloom. *Environ. Sci. Technol.* **47**, 8999–9013 (2013).

31. Griffiths, D. J. & Saker, M. L. The Palm Island Mystery Disease 20 Years on: A Review of Research on the Cyanotoxin Cylindrospermopsin. *Env. Toxicol* **18**, 78–93 (2003).

32. Chorus, I., Falconer, I. R., Salas, H. J. & Bartram, J. Health risks caused by freshwater cyanobacteria in recreational waters. *J. Toxicol. Environ. Heal. Part BCritical Rev.* **3**, 323–347 (2000).

33. Messineo, V., Melchiorre, S., Di Corcia, A., Gallo, P. & Bruno, M. Seasonal succession of Cylindrospermopsis raciborskii and Aphanizomenon ovalisporum blooms with cylindrospermopsin occurrence in the volcanic Lake Albano, Central Italy. *Environ. Toxicol.* **25**, 18–27 (2010).

34. WHO. *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. (E & FN Spon, 1999).

35. OPCW. *Organisation for the Prohibition of Chemical Weapons: Convention on the prohibition of the development, production, stockpiling and use of chemical weapons and on their destruction.* (2005).

36. WHO. *WHO recommendations on scientific, analytical and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC)*. (2018).

37. Macário, I. P. E. *et al.* Correction to: Stepwise strategy for monitoring toxic cyanobacterial blooms in lentic water bodies (Environ Monit Assess, 10.1007/s10661-017-6292-9). *Environ. Monit. Assess.* **189**, 620 (2017).

38. Jančula, D. & MarŠálek, B. Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. *Chemosphere* **85**, 1415–1422 (2011).

39. Visser, P. M., Ibelings, B. W., Bormans, M. & Huisman, J. Artificial mixing to control cyanobacterial blooms: a review. *Aquat. Ecol.* **50**, 423–441 (2016).

40. Newcombe, G. *International Guidance Manual for the Management of Toxic Cyanobacteria*. (2009).

41. Becker, A., Herschel, A. & Wilhelm, C. Biological effects of incomplete destratification of hypertrophic freshwater reservoir. *Hydrobiologia* **559**, 85–100 (2006).

42. Chen, S. *et al.* Effectiveness of a bubble-plume mixing system for managing phytoplankton in lakes and reservoirs. *Ecol. Eng.* **113**, 43–51 (2018).

43. Nürnberg, G. K., LaZerte, B. D. & Olding, D. D. An artificially induced planktothrix rubescens surface bloom in a small kettle lake in Southern ontario compared to blooms world-wide. *Lake Reserv. Manag.* **19**, 307–322 (2003).

44. Bakker, E. S. & Hilt, S. Impact of water-level fluctuations on cyanobacterial blooms: options for management. *Aquat. Ecol.* **50**, 485–498 (2016).

45. Paerl, H. W. *et al.* Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. *Harmful Algae* **54**, 213–222 (2016).

46. Cairns, M. J., Krauss, C. & Johnston, J. H. Recovery of phosphate from surface waters using a calcium silicate composite material for potential application in environmental remediation. *TechConnect Briefs* **3**, 252–255 (2013).

47. Nakamura, H. Water chemical remediation for simultaneous removal of phosphate ion and blue-green algae from anthropogenically eutrophied pond. in *Water Chemistry* 1–17 (2019). doi:http://dx.doi.org/10.5772/57353

48. Nie, M. *et al.* Simultaneous removal of bisphenol A and phosphate from water by peroxymonosulfate combined with calcium hydroxide. *Chem. Eng. J.* **369**, 35–45 (2019).

49. Matthijs, H. C. P. *et al.* Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Res.* **46**, 1460–1472 (2012).

50. Ho, L. *et al.* Fate of cyanobacteria and their metabolites during water treatment sludge management processes. *Sci. Total Environ.* **424**, 232–238 (2012).

51. Wang, X., Wang, X., Wei, Z. & Zhang, S. Potent removal of cyanobacteria with controlled release of toxic secondary metabolites by a titanium xerogel coagulant. *Water Res.* **128**, 341–349 (2018).

52. Ahmad, A. L., Mat Yasin, N. H., Derek, C. J. C. & Lim, J. K. Optimization of microalgae coagulation process using chitosan. *Chem. Eng. J.* **173**, 879–882 (2011).

53. Wang, L. *et al.* Flocculation of Microcystis aeruginosa using modified larch tannin. *Environ. Sci. Technol.* **47**, 5771–5777 (2013).

54. House, J. & Burch, M. *Using algicides for the control of algae in Australia*. (2002).

55. Lin, Y. *et al.* Cyanobacterial bloom mitigation by sanguinarine and its effects on aquatic microbial community structure. *Environ. Pollut.* **253**, 497–506 (2019).

56. Gragnani, A., Scheffer, M. & Rinaldi, S. Top-down control of cyanobacteria: A theoretical analysis. in *American Naturalist* **153**, 59–72 (1999).

57. Demeke, A. Cyanobacteria blooms and biological control methods. *Int. J. Fauna Biol. Stud.* **3**, 32–38 (2016).

58. Mohamed, Z. A. Macrophytes-cyanobacteria allelopathic interactions and their implications for water resources management - A review. *Limnologica* **63**, 122–132 (2017).

59. Ahn, C. *et al.* Selective control of cyanobacteria by surfactin-containing culture broth of Bacillus subtilis C1. *Biotechnol. Lett.* **25**, 137–142 (2003).

60. Manage, P. M., Kawabata, Z. & Nakano, S. ichi. Algicidal effect of the bacterium Alcaligenes denitrificans on Microcystis spp. *Aquat. Microb. Ecol.* **22**, 111–117 (2000).

61. Cannon, R., Shane, M. & Whitaker, J. Interaction of Plectonema boryanum (Cyanophyceae) and the LPP-cyanophages in continuous culture. *J. Phycol.* **12**, 418–421 (2008).

62. Gerphagnon, M., Colombet, J., Latour, D. & Sime-Ngando, T. Spatial and temporal changes of parasitic chytrids of cyanobacteria. *Sci. Rep.* **7**, 1–9 (2017).

63. Waterbury, J. & Valois, F. Resistance to co-occurring phages enables marine Synechococcus communities to coexist with cyanophages abundant in seawater. *Appl. Environ. Microbiol.* **59**, 3393–3399 (1993).

64. Dryden, R. & Wright, S. Predation of cyanobacteria by protozoa. *Can. J. Microbiol.* **33**, 471–482 (1987).

65. Atkins, R., Rose, T., Brown, R. S. & Robb, M. The Microcystis cyanobacteria bloom in the Swan River - February 2000. *Water Sci. Technol.* **43**, 107–114 (2001).

66. Kozacek, C. Circle of blue. (2014). Available at: https://www.circleofblue.org/2014/world/draft-world-stands-algae-dead-zones-ruin-water/. (Accessed: 4th February 2020)

67. Wijffels, R. H., Kruse, O. & Hellingwerf, K. J. Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. *Curr Opin Biotechnol* **24**, 405–413 (2013).

68. Raheem, A., Prinsen, P., Vuppaladadiyam, A. K., Zhao, M. & Luque, R. A review on sustainable microalgae based biofuel and bioenergy production: Recent developments. *J. Clean. Prod.* **181**, 42–59 (2018).

69. Burja, A. M. *et al.* Marine cyanobacteria: a profilic source of natural products. *Tetrahedron* **57**, 9347–9377 (2001).

70. Koller, M., Muhr, A. & Braunegg, G. Microalgae as versatile cellular factories for valued products. *Algal Res.* **6**, 52–63 (2014).

71. Cardozo, K. H. M. *et al.* Metabolites from algae with economical impact. *Comp. Biochem. Physiol.*

Part C **146**, 60–78 (2007).

72. Olaizola, M. Commercial production of astaxanthin from Haematococcus pluvialis using 25,000-liter outdoor photobioreactors. *J. Appl. Phycol.* **12**, 499–506 (2000).

73. Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A. Commercial applications of microalgae. *J. Biosci. Bioeng.* **101**, 87–96 (2006).

74. Hosikian, A., Lim, S., Halim, R. & Danquah, M. K. Chlorophyll extraction from microalgae: A review on the process engineering aspects. *Int. J. Chem. Eng.* 1–11 (2010). doi:10.1155/2010/391632

75. Díaz, G. D., Li, Q. & Dashwood, R. H. Caspase-8 and Apoptosis-inducing Factor Mediate a Cytochrome c-independent Pathway of Apoptosis in Human Colon Cancer Cells Induced by the Dietary Phytochemical Chlorophyllin. *Cancer Res.* **63**, 1254–1261 (2003).

76. Karmali, R. A. Historical perspective and potential use of n-3 fatty acids in therapy of cancer cachexia. *Nutrition* **12**, S2–S4 (1996).

77. Horimoto, N. & Ogawa, T. Arachidonic Acid Activation of Potassium Channels in Rat Visual Cortex Neurons. *Neuroscience* **77**, 661–671 (1997).

78. Fan, Y. Y. & Chapkin, R. S. Importance of Dietary gamma-Linolenic Acid in Human Health and Nutrition. *J Nutr* **128**, 1411–1414 (1998).

79. Holub, B. J. Docosahexaenoic acid (DHA) and cardiovascular disease risk factors. *Prostaglandins Leukot. Essent. Fat. Acids* **81**, 199–204 (2009).

80. Ytrestøyl, T., Aas, T. S. & Åsgård, T. Utilisation of feed resources in production of Atlantic salmon (Salmo salar) in Norway. *Aquaculture* **448**, 365–374 (2015).

81. FAO/WHO. *Fats and oils in human nutrition*. (1993).

82. Echeverría, F., Valenzuela, R., Catalina Hernandez-Rodas, M. & Valenzuela, A. Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: New dietary sources. *Prostaglandins Leukot. Essent. Fat. Acids* **124**, 1–10 (2017).

83. Tiwari, O. N. *et al.* Characterization and optimization of Bioflocculant exopolysaccharide production by Cyanobacteria Nostoc sp. BTA97 and anabaena sp. BTA990 in culture conditions. *Appl. Biochem. Biotechnol.* **176**, 1950–1963 (2015).

84. De Philippis, R. & Vincenzini, M. Exocellular polysaccharides from cyanobacteria and their possible applications. *FEMS Microbiol. Rev.* **22**, 151–175 (1998).

85. Mishima, T. *et al.* Inhibition of tumor invasion and metastasis by calciumspirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, Spirulina platensis. *Clin. Exp. Metastasis* **16**, 541–550 (1998).

86. Shah, V., Ray, A., Garg, N. & Madamwar, D. Characterization of the Extracellular Polysaccharide

Produced by a Marine Cyanobacterium, Cyanothece sp. ATCC 51142, and Its Exploitation Toward Metal Removal from Solutions. *Curr. Microbiol.* **40**, 274–278 (2000).

87. Bhati, R., Samantaray, S., Sharma, L. & Mallick, N. Poly-β-hydroxybutyrate accumulation in cyanobacteria under photoautotrophy. *Biotechnol. J.* **5**, 1181–1185 (2010).

88. Koller, M., Gasser, I., Schmid, F. & Berg, G. Linking ecology with economy: Insights into polyhydroxyalkanoate-producing microorganisms. *Eng. Life Sci.* **11**, 222–237 (2011).

89. Gradíssimo, D. G., Xavier, L. P. & Santos, A. V. Cyanobacteria polyhydroxyalkanoates: a sustainable alternative in circular economy. *Molecules* **25**, 4331 (2020).

90. Kultschar, B. & Llewellyn, C. Secondary Metabolites in Cyanobacteria. in *Secondary Metabolites - Sources and Application* (eds. Vijayakumar, R. & Raja, S. S. S.) 23–36 (2018). doi:10.5772/intechopen.75648

91. Fawzy, M. A. & Mohamed, A. K. S. H. Bioremediation of heavy metals from municipal sewage by cyanobacteria and its effects on growth and some metabolites of Beta vulgaris. *J. Plant Nutr.* **40**, 2550–2561 (2017).

92. Grzesik, M. & Kalaji, H. M. Effectiveness of cyanobacteria and green algae in enhancing the photosynthetic performance and growth of willow (Salix viminalis L.) plants under limited synthetic fertilizers application. *Photosynthetica* **55**, 510–521 (2017).

93. Lengke, M. F., Fleet, M. E. & Southam, G. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver(I) nitrate complex. *Langmuir* **23**, 2694–2699 (2007).

94. Zhao, J., Li, X.-F., Ren, Y.-P., Wang, X.-H. & Jian, C. Electricity generation from Taihu Lake cyanobacteria by sediment microbial fuel cells. *J. Chem. Technol. Biotechnol.* **87**, 1567–1573 (2012).

95. Vasudevan, V. *et al.* Environmental performance of algal biofuel technology options. *Environ. Sci. Technol.* **46**, 2451–2459 (2012).

96. Rosa, I. C., Costa, R., Gonçalves, F. & Pereira, J. L. Bioremediation of metal-rich effluents: could the invasive bivalve work as a biofilter? *J. Environ. Qual.* **43**, 1536–1545 (2014).

97. Domingues, A. *et al.* Potential of the bivalve Corbicula fluminea for the remediation of olive oil wastewaters. *J. Clean. Prod.* **252**, 119773 (2020).

98. Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **25**, 294–306 (2007).

99. Rose, P. D. *et al. Salinity, Sanitation and Sustainability: a study in environmental biotechnology and integrated wastewater benefication in South Africa*. **3**, (2007).

100. Nagy, B. J. *et al.* MAB2.0 project: Integrating algae production into wastewater treatment. *EuroBiotech J.* **2**, 10–23 (2018).

101. Levi, D. Adressing an ecological crisis. (2018). Available at: https://aecom.com/blog/addressing-an-

ecological-crisis. (Accessed: 23rd October 2020)

102. Pearson, L. A. & Neilan, B. A. The molecular genetics of cyanobacterial toxicity as a basis for monitoring water quality and public health risk. *Curr. Opin. Biotechnol.* **19**, 281–288 (2008).

103. Tillett, D., Parker, D. L. & Neilan, B. A. Detection of toxigenicity by a probe for the microcystin synthetase A gene (mcyA) of the cyanobacterial genus Microcystis: comparison of toxicities with 16S rRNA and phycocyanin operon (Phycocyanin Intergenic Spacer) phylogenies. *Appl. Environ. Microbiol.* **67**, 2810–2818 (2001).

104. Schembri, M. A., Neilan, B. A. & Saint, C. P. Identification of Genes Implicated in Toxin Production in the Cyanobacterium Cylindrospermopsis raciborskii. *Environ. Toxicol.* **16**, 413–421 (2001).

105. Al-Tebrineh, J., Kaan Mihali, T., Pomati, F. & Neilan, B. Detection of saxitoxin-producing cyanobacteria and Anabaena circinalis in environmental water blooms by quantitative PCR. *Appl. Environ. Microbiol.* **76**, 7836–7842 (2010).

106. Wood, S. A., Heath, M. W., Kuhajek, J. & Ryan, K. G. Fine-scale spatial variability in anatoxin-a and homoanatoxin-a concentrations in benthic cyanobacterial mats: implication for monitoring and management. *J. Appl. Microbiol.* **109**, 2011–2018 (2010).

107. Koskenniemi, K., Lyra, C., Rajaniemi-Wacklin, P., Jokela, J. & Sivonen, K. Quantitative Real-Time PCR Detection of Toxic Nodularia Cyanobacteria in the Baltic Sea. *Appl. Environ. Microbiol.* **73**, 2173–2179 (2007).

108. Hiskia, A., Spoof, L., Kaloudis, T. & Meriluoto, J. Determination of Cyanotoxins by High-Performance Liquid Chromatography with Photodiode Array. in *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis* (eds. Meriluoto, J., Lisa, S. & Codd, G. A.) (John Wiley & Sons, Ltd., 2017). doi:10.1002/9781119068761.ch21

109. Oehrle, S. A., Southwell, B. & Westrick, J. Detection of various freshwater cyanobacterial toxins using ultra-performance liquid chromatography tandem mass spectrometry. *Toxicon* **55**, 965–972 (2010).

110. Sanseverino, I., António, D. C., Loos, R. & Lettieri, T. *Cyanotoxins: methods and approaches for their analysis and detection. JRC Technical Reports* (2017).

111. OECD. Glossary of statistic terms. (2001). Available at: https://stats.oecd.org/glossary/detail.asp?ID=2827. (Accessed: 26th October 2020)

112. Martins, M. *et al.* Sustainable strategy based on induced precipitation for the purification of phycobiliproteins. *ACS Sustain. Chem. Eng.* **submitted**, (2021).

113. Passos, H., Freire, M. G. & Coutinho, J. A. P. Ionic liquid solutions as extractive solvents for value-added compounds from biomass. *Green Chem.* **16**, 4786–4815 (2014).

114. Sintra, T. E. *et al.* Sequential recovery of C-phycocyanin and chlorophylls from Anabaena cylindrica.

Sep. Purif. Technol. **255**, 117538 (2021).

115. Rito-Palomares, M., Nuez, L. & Amador, D. Practical application of aqueous two-phase systems for the development of a prototype process for c-phycocyanin recovery from Spirulina maxima. *J. Chem. Technol. Biotechnol.* **76**, 1273–1280 (2001).

116. Slegers, P. M. *et al.* Design of Value Chains for Microalgal Biorefinery at Industrial Scale: Process Integration and Techno-Economic Analysis . *Frontiers in Bioengineering and Biotechnology* **8**, 1048 (2020).