PERSPECTIVE

Low doses of toxicants can enhance algae potential as biodiesel and biomass feedstocks

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ABSTRACT

The ambitious target of worldwide governments for achieving carbon neutrality by the middle of the current century confers a key role to negative emission technologies. To meet this target, algae-based renewable bioenergy is expected to find larger-scale application. However, the photosynthetic efficiency and potential of algae to produce biomass and biofuel should be improved, and further bioengineering developments are needed. Considerable evidence has recently accumulated to show that a plethora of toxicants stimulate algae at low doses (hormesis), an effect that is controversial to the long-held belief that toxicants only suppress algae at high doses. Low doses of toxicants induce mild oxidative stress, which increases the synthesis of photosynthetic pigments and thus sunlight capturing potential. Photosynthesis is enhanced, and algal growth and biomass also increase. Protein content, total lipids, and biochemical quality are also increased in a dose-dependent manner, indicating the potential of low-dose stress to enhance algal biomass and biofuel. Underlying molecular mechanisms driving these uncovered low-dose-stress responses started to be unraveled, providing an opportunity for novel bioengineering developments to maximize algal potential as feedstock for the production of carbon-neutral fuel. Further scientific developments are needed to improve the performance of algae as a feedstock, reduce the cost of their cultivation, and enhance their resilience in toxicant-

containing wastewater. These also call for reduction of toxicants to low levels adequate for improved performance of algae cultured for bioenergy feedstock.

Keywords: bioenergy; biodiesel; renewable energy; microalgae; hormetic response

List of abbreviations
ABC transporters: proteins regulating the ATP-powered substance transportation across membranes
CO_2: carbon dioxide
D1: protein synthesized as a precursor with a short C-terminal extension
EES: electrochemical energy storage
MAX: maximum stimulatory response to low-dose stress
mcm2: Minichromosome Maintenance Complex Component 2 gene
mcm6: Minichromosome Maintenance Complex Component 6 gene
mRNA: messenger RNA
NOAEL: no-observed-adverse-effect-level
NPQ: non-photochemical fluorescence quenching
O_2: oxygen
prim2: DNA Primase Subunit 2 gene
psbA: gene involved in the synthesis of the D1 protein of PSII reaction center
PSII: photosystem II
ROS: reactive oxygen species
R&D: research and development
VOCs: volatile organic compounds
2,4-D: 2,4-dichlorophenoxyacetic acid

1. Introduction

Carbon neutrality by the middle of the 21st century is the top urgent mission of the world [1,2]. Negative emission technologies are of utmost importance to remove CO_2 from the atmosphere and facilitate the achievement of the ambitious target of carbon neutrality by the middle of the century [3]. Bioenergy with carbon capture and storage can contribute to CO_2 mitigation [3]. To neutralize CO_2 emissions and attain net-zero-CO_2 societies, hydrogen economy should be deployed, and large-scale algae production for bioenergy may contribute toward this attainment [4,5].

Algal biomass has attracted interest as feedstock for the production of carbon-neutral fuel because of the advantages that algae offer. These include high photosynthetic efficiency, lipid productivity, growth rate, and biomass yield, as well as cultivation in controlled, artificial ponds, not requiring arable land [3,6–9]. Algae can be approximately ten times more efficient in converting solar energy to biomass than terrestrial plants, and algae-based CO_2 removal represents a negative CO_2 emission path [3]. For the annual production of 100 metric tons of algal biomass, 183 metric tons of CO_2 can be consumed [8]. Algae can also utilize sunlight to produce lipids more efficiently than higher crop plants [8]. Hence, algae are considered feedstock for a new-generation of biofuels [9,10]. Algae are also used for a number of applications in materials science, for example as a renewable source for the development of electrochemical energy storage (EES) devices to reduce environmental pollution [11]. Therefore, algae play a multi-dimensional role in the area of renewable energy.

Transition from first-generation biofuels to higher-generation biofuels, including algal fuel, may be even more advantageous in recognition of the current challenges facing the world, especially amid the Ukraine-Russia conflict and its unfavorable effects on fuel and energy supply chain [12]. Considering the superiority of algae in converting energy to biomass to terrestrial plants [3], algal cultivation may also contribute in decreasing ground-level ozone pollution, which was also widely worsened in worldwide cities due to movement restriction measures implemented during the COVID-19 pandemic [13–15]. This is because higher plants cultivated for renewable energy, such as poplars, are high emitters of ozone-forming volatile organic compounds (VOCs), especially the most abundant isoprene [16–19]. Decreasing VOCs emissions from bioenergy cultivations can lower ozone concentrations and further prevent adverse effects on human health and mortalities as well as loss of carbon biomass due to ozone-induced stress in higher plants [18,20–22]. Therefore, algal-based renewable energy can play an important role in achieving carbon neutrality given the recent developments around the globe.

Various technologies exist or are under development to produce biomass and liquid fuel from algae, and reducing the cost of such fuel production is of high interest [6,23,24]. However, the photosynthetic efficiency and biomass and biofuel productivity and quality of biofuel-producing algae should be maximized, such as through algal bioengineering [10]. Moreover, algae cultivated for commercial biomass and biofuel production should also be tolerant to toxicants contained in wastewater or reused water [24,25].

Hormesis is a biphasic dose response which includes potentially beneficial effects of low-dose toxicants and adverse effects of higher-dose toxicants. Low doses activate *adaptive response* channels, preparing organisms to cope with oxidative stress, stimulating them, and eventually enhancing their performance under stress conditions [26–33]. In plants, hormetic response in photosystem II (PSII) is triggered by mild increases in reactive oxygen species (ROS) and regulation of the non-photochemical fluorescence quenching (NPQ) of photosynthesis, which dissipates excess light energy and protects against excessive ROS accumulation [27]. These mechanisms also involve the signalling action of hydrogen peroxide and promotion of the malleability of cell walls causing cell expansion, often promoting plant development and enhancing growth and productivity under low-dose stress [34,35]. Therefore, understanding hormesis in algae can offer a biological platform to maximize energy conversion and biomass and biofuel productivity as well as to select toxicant-tolerant genotypes or strains.

Moreover, it can facilitate the identification of new genes, proteins, and pathways, activated by such low doses, which can lead to improved bioengineering applications to maximize algal biomass and biofuel production efficiency. Besides, algal hormesis can guide programs aiming at revealing the limits of toxicants to which certain genotypes or strains can perform better or tolerate. Hence, this article evaluates published literature reporting hormetic responses of algae to toxicants to provide support for the general occurrence of hormesis across algae and offer a perspective for its potential utilization in the cultivation of algae as feedstock for renewable bioenergy. The concept of hormesis and its relevance to the performance of algal cultivation as feedstock for biomass and biofuel are illustrated in Figure 1.

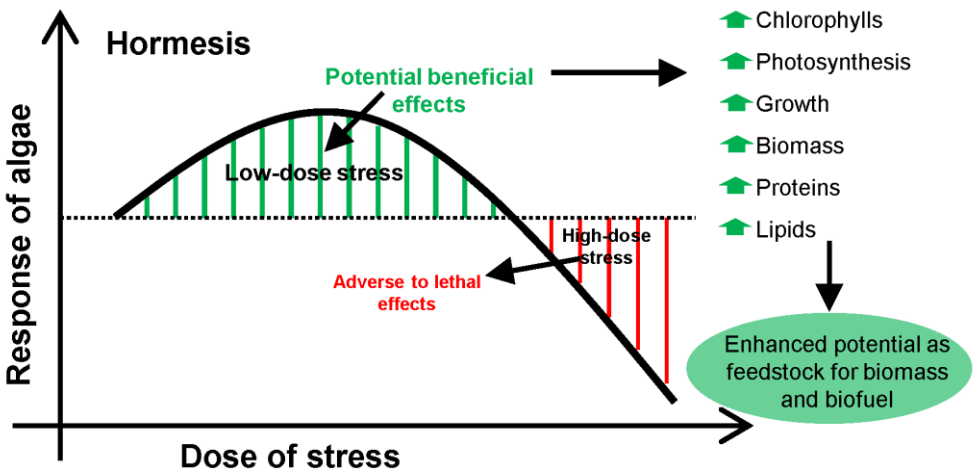

Figure 1. A conceptual diagram of hormetic response. The traditional toxicological threshold is also called no-observed-adverse-effect-level (NOAEL).

1. Analysis of the issue

Ample evidence has accumulated to suggest the widespread induction of hormesis in algae by toxicants (Table 1). For instance, extensive screenings also revealed 6 hormetic responses of growth to different agrochemicals in three freshwater microalgae species (green alga *Desmodesmus subspicatus* and the diatoms *Nitzschia palea* and *Navicula pelliculosa*) [38]. A further literature survey led to the identification of hormesis induced by allelopathic algicides (e.g. juglone) [39,40], antibiotics [41–48], different bisphenol congeners [49], effluents from textile-dyeing wastewater treatment plants [50], humic susbtances [51], (micro)plastics and various leachates [52–54], nanoparticles [55], and nanoscale bismuth oxyiodide [56]. Algal hormesis was also induced by polycyclic aromatic hydrocarbons [57], quaternary ammonium cationic surfactants [58], rare earth elements [59], and various classes of pesticides [60–62]. These extensive evaluations demonstrate that numerous toxicants of diverse mode of actions and widespread presence in the global environmental media induce hormesis in algae, suggesting that the occurrence of algal hormesis does not depend on the type of chemical.

Table 1. Quantitative estimates of hormesis induced by different toxicants in algae. MAX-NOAEL: distance from the maximum stimulatory response (MAX) to the no-observed-adverse-effect-level (NOAEL). n/a: not available.

Algal organisms	Toxicants	Endpoints	Stimulation frequency	Stimulation magnitude	MAX-NOAEL	Reference
Raphidocelis subcapitata (previously *Pseudokirchneriella subcapitata*)	Seven herbicides	Growth	Treatment exceeded the control levels in 56% of the dose-response curves; while 23% of the dose-response curves[1] were described better by a model including hormesis	Average = 16 ±16%	8 ±4 fold	[36]
Various algae	Various nanomaterials	All endpoints pooled[3]	n/a	MAX=23.4 ±2.8%	4.1 ±1.0 fold	[37][2]

[1]out of 77 dose-response curves that satisfied the criteria for evaluation of hormesis.
[2]the estimates reported here are based on a re-analysis of the published database with hormetic responses of algae. The MAX is based on 46 dose responses, whereas the MAX-NOAEL is based on 39 dose-response curves.
[3]The dose responses concerned an array of endpoints; however, 52.2% of the dose responses concerned growth, biomass, photosynthesis-related endpoints, and survival

Hormetic responses to toxicants were found in numerous algae, including the cosmopolitan diatom *Asterionella formosa* [39], the soil and freshwater unicellular *Chlamydomonas reinhardtii* [56], different species of green unicellular microalgae *Chlorella* sp.[40,42–44,49,53,55,58,62,63] and freshwater green algae *Scenedesmus* sp. [48,50,57,60], and the marine microalga *Dunaliella tertiolecta* [52]. Such responses were also observed in *D. subspicatus* [49], the commonly used for oil production *Synechocystis* sp. [45,46], the freshwater unicellular alga *Euglena gracilis* [59,61], the freshwater diatom *Fragilaria crotonensis* [39], and the microalga *R. subcapitata* [41,42,47]. Examples of hormesis induced in bioenergy-relevant algae from the published literature are illustrated in Figure 2. These findings suggest that toxicant-induced hormesis occurs across algal species, including species and genera representative of some of the most commercially important algae that are also most cultivated in wastewater because of their relatively high biomass, tolerance, and lipid yield. For example, this is the case for *Chlorella* and *Scenedesmus* genera [64–67], or for *C. reinhardtii* [56], which is also an excellent model for biofuel and bioproduct production [68]. The tolerance or resistance of such species to high doses of pollutants/stresses[65] can now be explained by hormetic processes, which ultimately lead to increased tolerance of such organisms to toxicants [69].

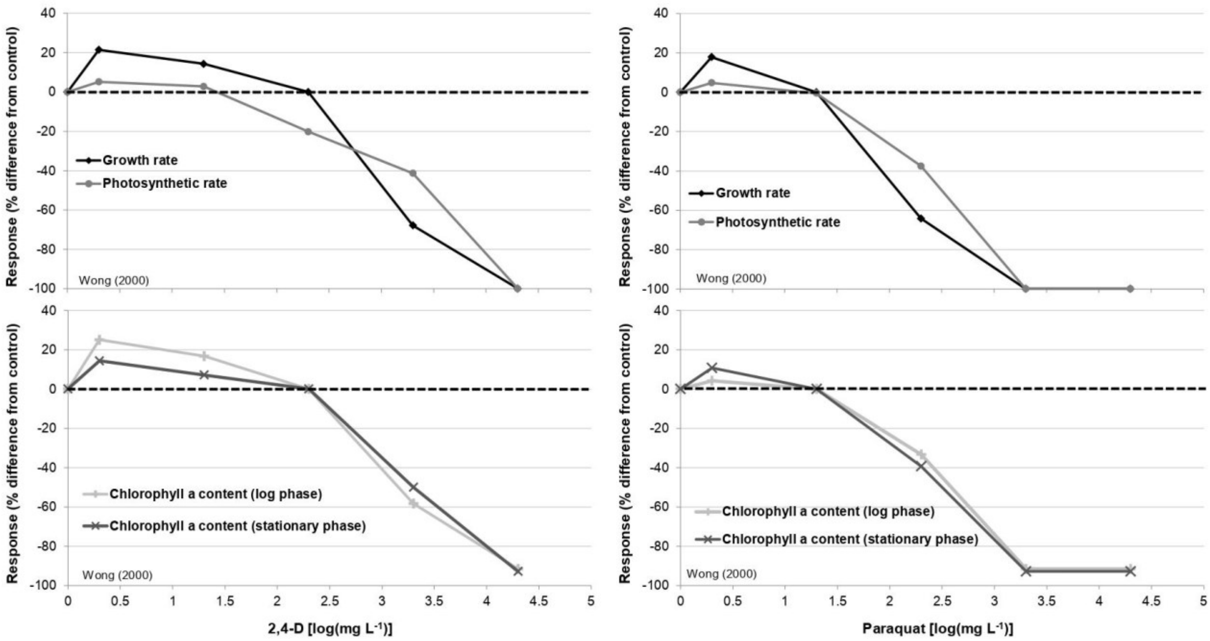

Figure 2. Examples of hormetic-like responses in *Scenedesmus quadricauda* Berb 614, a promising microalga for bioenergy production and animal food, grown in the presence or absence of

herbicides. The actual concentrations of paraquat and 2,4-dichlorophenoxyacetic acid (2,4-D) were 0 (control), 0.02, 0.2, 2, 20, and 200 mg L^{-1}. The concentrations were log-transformed for presentation clarity. Data were extracted from Wong et al. [60] According to the statistical analyses results reported by Wong et al. [60], as many as eleven positive low-dose responses were significant.

Hormetic responses of algae were mainly observed in chlorophyll synthesis [44–46,48,50,54,58,60,62], photosynthetic rate and activity [44–46,54,60], biomass [46,59,63], and cell density/growth [39–47,49,50,52–54,56–58,60–62]. Hormesis occurred independently of log or stationary phase of algae growth [60]. In these studies, the low doses causing stimulation were commonly multi-fold smaller than the smallest doses causing inhibition, even 1000 times smaller (Fig. 2) [47,56,59,60]. The magnitude of the stimulation was commonly smaller than two-fold the control response, and typically not exceeding 60%, in agreement with the broad hormesis literature [70,71].

Toxicants can also enhance the protein content, total lipids, and biochemical quality in a dose-dependent manner [44,59,63,72,73]. Changes in the fatty acid composition by sub-NOAEL doses also occur, which may improve biofuel combustion performance [45,46,55]. This hypothesis is now validated in a recent study, including also real-environment water, that showed that *E. gracilis* could remediate rare earth elements from contaminated acidic water, with low doses of contaminants increasing its growth and biodiesel productivity [59]. In a follow-up assay, contaminant treatment and fermentation of wax ester improved the biodiesel quality [59]. Our literature review also suggests that hormetic responses can affect the growth and quality of algae used medicinally or for dietary supplements. In the application for biofuel production, toxins are burned out/oxidized. However, toxicant-induced hormesis in medicinal or otherwise consumed algae raises concerns about toxicants and harmful byproducts of metabolism entering the food chain [42–44,55,58,62,63,74]. Since toxicant-induced hormesis also affects algae that are promising sources of bioenergy as well as their lipid productivity, such hormetic responses can increase lipid productivity and enhance algae-based bioenergy [42,45,46,55,58,59,62,63]. Therefore, hormetic responses represent a double-edge sword. They can be utilized to enhance the amount and quality of algae used for bioenergy in controlled environments (by exposing them to non-toxic stresses), but may also undermine the quality and value of algae exposed to toxicants in non-controlled environments (nature) and introduce human health risks.

Although the molecular underlying mechanisms remain unclear, recent studies now shed light at molecular level. In particular, the hormetic response of the growth of the microalga *R. subcapitata* to the antibiotic erythromycin occurred in tandem with a similar pattern in the up- and down-regulation of genes enriched in DNA replication process (e.g. *mcm2*, *mcm6*, *pri2*), suggesting that DNA replication process may be a major determinant of hormesis at the algae population level [41]. For the same species, another recent metabolomic study also revealed that algal growth stimulation by a low dose of erythromycin (4 μg L^{-1}) was linked to various metabolic pathways, such as ABC transporters, purine metabolism, and fatty acid biosynthesis, highlighting that low doses drive energy metabolism [47]. These responses were opposite to the dysregulation of metabolic pathways linked to growth inhibition by 80 and 120 μg L^{-1} [47]. Furthermore, the hormetic effect of the antibiotic azithromycin on the microalga *C. pyrenoidosa* was characterized by enhanced activity of the PSII reaction center due to up-regulation of mRNA expression of the gene *psbA*, which is involved in the synthesis of the D1 protein of PSII reaction center [44]. Enriched carotenoids and chlorophyll *b* could enhance the absorption of light energy, reduce the oxidative damage, and contribute to increasing proteins, carbohydrates, and lipids [44]. Further studies exposing *Synechocystis* sp. to sulfamethoxazole and tetracycline antibiotics show that the low-dose stimulation is driven by the up-regulation of numerous proteins related to differentiation and division of cells, and gene expression, and photosynthesis, and down-regulation of proteins regulating the catabolism and transport of carbohydrates [45].

2. Future outlook and perspectives

These recent scientific advances provide a knowledge platform regarding the mechanisms underlying the stimulation of algae by low-dose stress that can be transferred into bioengineering to enhance the performance of algae cultivated as bioenergy feedstock. However, these advances also illustrate the important need of further research efforts in this area, especially because these new understandings provide the basis for newly emerged questions. For example, how does stimulation by low stress doses affect the biochemical composition of algal biomass in detail? The biomass biochemical composition affects the economics associated with algal cultivation as a biofuel feedstock, with enriched lipids content leading to decreases in other valuable biochemicals in the biomass [9]. Can the concept of stimulation by low-dose stress be utilized to increase the content of algal oil without decreasing or even while enriching other valuable biochemicals in the biomass? These are some important questions, which to be answered a dose-time-response component should be considered due to the temporal variation in hormetic responses [48,49,56,59,60].

Despite the unanswered questions, however, these advances highlight that hormetic responses can be utilized as a tool to potentially enhance microalgae for optimizing a sustainable and renewable source of biofuels, feed, and other useful products of atmospheric CO_2 conversion, such as lipids, carbohydrates, and other bioactive metabolites [75]. These advancements also illustrate the potential real-world applicability of the hormesis concept to improve bioremediation of contaminated waters while also improving biodiesel productivity and quality [59]. This is important because biodiesel can lead to more sustainable transportation fuels, warranting further enhancement of production efficiency and economic viability [76–78]. Application of post-genome tools to algae can solve important bottlenecks in R&D of algae-derived biofuel [10]. The current scientific base suggests the potential of metabolic engineering of algal O_2-generating photosynthesis based on low-dose stress mechanisms to facilitate the fossil fuel replacement and decrease the effect on the greenhouse gas inventory in the atmosphere due to the current methods of biofuel production [10]. Hormetic mechanisms can also enlighten the scientific and technological developments in semi-artificial photosynthesis [79], by revealing previously unidentified molecules and mechanisms that are activated by low doses of stress to ultimately enhance light capturing, energy conversion and flow, and biological carbon fixation [27]. Finally, it is important to understand and incorporate these hormetic responses because of the wide presence of toxicants in (reused) water. Such toxicants often exist at low concentrations, due to the inadequacy and high costs of existing technologies to fully remove contaminants [80], which may pose risks to humans and other organisms consuming algae affected by toxicants in nature. These can lead to the development of new algal genotypes with higher photosynthetic efficiencies and relatively high tolerance toward toxicants in recognition of algae cultivation in wastewater [24,25]. Screenings incorporating hormesis can provide a cost-benefit analysis medium for deciding the 'acceptable' toxicant levels in the cultivations of artificial algal cultures while maximizing the biomass and biofuel productivity and quality. Acceptable levels in artificial cultures for biofuel production would be those not adversely affecting but even enhancing algal growth and biofuel quality. Conversely, in uncontrolled environments, where algae are not cultured for biofuel, acceptable levels would be those not creating ecological and human health risks if such algae are ingested by any means. Hence, incorporation of hormesis becomes a precondition for accurate cost-benefit analyses.

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Authors contributions: E.A. designed the study, reviewed the literature, drafted the manuscript, had a leading role, and supervised the production of the manuscript. J.G. and J.P. reviewed the manuscript and contributed intellectual input. All authors approved the final version for publication.

Declaration of Conflicting Interests The authors declare that there is no conflict of interest.

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