Review

Antioxidant Production in *Dunaliella*

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Abstract: Microalgae have become an attractive natural source of a diverse range of biomolecules, including enzymatic and non-enzymatic antioxidants; nevertheless, economically sustainable production of such compounds from microalgae biomass is still challenging. The main hurdles are: (a) increasing microalgae yield; (b) achieving optimal cultivation conditions; (c) energy-efficient and cost-effective downstream processing (extraction and purification); (d) optimal storage of post-processed antioxidant molecules. This review provides a detailed overview of enzymatic and non-enzymatic antioxidants in the cellular metabolism of the commercially important microalgae *Dunaliella*, industrial applications of antioxidant enzymes, strategies to enhanced antioxidant accumulation in cells, and the opportunities and limitations of current technologies for antioxidant enzymes production from microalgae biomass as an alternative to common microbial sources.

Keywords: *Dunaliella*; antioxidant enzymes; cultivation conditions; post-harvest processing

1. Introduction

Microalgae are single celled micro-bio-factories capable of producing a wide variety of high-value compounds (carbohydrates, proteins, lipids, carotenoids, phycobiliproteins, phenolic, polyunsaturated fatty acids and antioxidants) used in the pharmaceutical, nutraceutical, cosmetic, and food processing industries [1–10]. Microalgal biomass can be added directly as a nutrient enhancer in animal feeds; as an enhancer for improving the quality of food; and as a stabiliser for maintaining the colour and flavour of food products [3,11–14]. The global microalgae market has gradually expanded, and worldwide sales are expected to exceed US $3.2 billion by the end of 2030 [15]. Recent studies have demonstrated the potential of microalgae to produce antioxidant molecules [16–19]. The antioxidant capacity and antioxidant activity of small molecules (β-carotene, astaxanthin, and phenolic compounds) have also been explored extensively [10,20,21]. Nevertheless, antioxidant enzymes have received little attention.

The green halotolerant microalgae *Dunaliella* can grow in a wide range of salt concentrations, from 0.05 M to 5.5 M NaCl [22,23]. Currently, 28 species of *Dunaliella* have been identified, of which 23 species live in saline environments, and 5 are rare species that inhabit freshwater [22]. Commercially, *Dunaliella* is cultivated in several countries, such as Australia, China, Israel, and India, with pilot-scale projects in Chile, Spain, Iran and Portugal [24–26] and considered one of the best sources of β carotene. *Dunaliella* has also been suggested as a sustainable source for industrial bioprocessing for the production of protein [27], biodiesel [4,28,29], colouring agent [30], and antioxidants [31].

Currently, antioxidant enzymes are sourced from microbial and animal sources [32,33]. Though there is no commercial production of antioxidant enzymes from *Dunaliella*, several reports have shown that *Dunaliella* can increase its levels of enzymatic (mainly catalase, peroxidase and superoxide dismutase) and non-enzymatic (carotenoids, phenolic, ascorbate and glutathione) antioxidants to mitigate stress induced by exogenous abiotic fac-
tors [31,34–39]. Its high adaptability to exogenous stressors (oxidative and osmosis) and the lack of a rigid cell wall [40] could make Dunaliella an ideal natural source for antioxidant production. Dunaliella can efficiently and sustainably produce large volumes of biomass without competing for cultivable land and fresh water [25].

2. Antioxidants

Extensive research on the effect of ROS in humans has demonstrated a substantial link between free radicals and more than sixty different health conditions, including ageing, cancer, diabetes, Alzheimer’s disease, strokes, heart attacks and atherosclerosis [41,42]. Consumption of higher levels of dietary antioxidant enzymes, as well as antioxidant molecules-enriched food or antioxidant supplements, has been found to reduce the risk of free radical-related health issues [41].

2.1. Classification of Antioxidants

Antioxidants (enzymatic or non-enzymatic) are classified depending on their mode of activity as primary antioxidants (hydrogen or electrons donors) or secondary antioxidants (oxygen scavengers or chelating agents) [43,44]. Antioxidants can also be grouped according to size, solubility, mode of action or structure, Figure 1. Abundant enzymatic antioxidants in microalgae are superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione transferase (GST). Non-enzymatic antioxidants consist of compounds such as Vitamin C (ascorbate), glutathione, carotenoid, phenolic compounds, proline, glycine, polyamine, PUFA, and some metals (Cu, Zu) [45–47]. Most enzymatic antioxidants and some non-enzymatic antioxidants (glutathione, ascorbate) are hydrophilic and mainly present in the cellular fluids (cytosol or cytoplasmic matrix), whereas the hydrophobic antioxidants (carotenoid, tocopherol) are primary located in the cell membranes [48].

![Classification of antioxidants](image)

**Figure 1.** Classification of antioxidants [41,44,48–50].

2.2. Antioxidant Enzymes

Antioxidant enzymes prevent or delay the oxidation of other molecules by neutralising reactive oxygen species (ROS) [48]. These enzymes eliminate ROS by reducing the energy of free radicals or by donating electrons to free radicals [42], and as such, constitute the first level of defence in the cells antioxidant network. Some molecules are not involved directly in the scavenging of free radicals but rather enhance other antioxidant molecules’ activity

[Image of classification diagram]

**Antioxidants**

- Based on the solubility
  - Water soluble antioxidants
  - Lipid soluble antioxidants

- Based on the mode of actions
  - Primary antioxidants
  - Secondary antioxidants

- Based on the structure
  - Enzymatic antioxidants
  - Non-enzymatic antioxidants
    - Phenolic
    - Flavonoids
    - Minerals
    - Thioles
    - PUFA (poly unsaturated fatty acids)
    - Vitamins
    - Carotenoid
    - Polysaccharides
    - Polyamines
and may also be classified as antioxidants [41]. The majority of antioxidant enzymes are metalloenzymes and contain a metal ion in their catalytic site [45].

2.3. Commercial Applications of Antioxidant Enzymes

Antioxidants, or antioxidant-enriched extracts, are commercially used to prevent oxidative processes and to maintain the flavor, texture, and colour of food during storage. They also find uses as refining, bleaching, deodorising agents in the food processing industries [51–55], in extending the shelf life of lubricating oil and reducing vehicular emissions [56] and in stabilisation of synthetic fibre, rubber, thermoplastic, and adhesives by stopping autocatalytic reactions [57]. In cosmeceutical products, antioxidant compounds are used to prevent skin ageing and UV-induced skin damage, and treat the appearance of wrinkles and erythema due to inflammation [58–61]. Industrial applications of antioxidants are listed in Table 1. Global demand for antioxidants was valued at ~USD 2.25 billion in 2014 and grew at a CAGR (compounds annual growth rate) of ~5.5% between 2015 and 2020 [62]. This increasing global demand is driving the search for synthetic and natural-derived antioxidants.

| Table 1. | | |
| --- | --- | --- |
| Natural Antioxidants | Applications | Ref |
| **SOD** | Added to cosmetic products to protect against skin damage |  |
| | Protect against lipid peroxidation, heat, and cold stress in poultry production |  |
| | As a therapeutic agent for treatment of inflammatory disorders |  |
| | Normal cells protector during radiotherapy for cancer patients |  |
| **CAT** | Eliminate excessive H2O2 in the textile industry, pulp, and paper industry |  |
| | used for bleaching fibres and pulp, and as a bactericidal disinfectant in food processing and in the pharmaceutical industry |  |
| | In aesthetics (mask treatment) to increase cellular oxygenation in the upper layers of facial epidermis |  |
| **GPX** | Reducing the risk of diabetes mellitus | [42,66] |
| **GST** | Protective role against neurogenerative diseases | [66,67] |
| | Decreases the risk of tumours of the head and neck, oral, cavity and colon |  |
| **Glutathione** | Anti-wrinkle formation, and as a modifier of skin smoothness |  |
| **Vitamins** | As a food preservative and bread improver, protective activity against heart diseases, reduced the risk of colorectal adenomas and prostate cancer, reduction of thyroid hormone levels | [69–73] |
| **Flavonoids** | As cancer preventive agents, protection against type 2 diabetes Functional food additive | [74–77] |
| **Carotenoids** | Anticancer agents, additive to cosmetics and multivitamin preparation |  |
| | Food colouring agent, pro-vitamin A in food and animal feed |  |
| **PUFA** | Prevention of heart and inflammatory diseases | [79,80] |
| **BHA** | Extending the shelf life of vegetable oil, frying oil, animal feed, cereals, chewing gum, potato flakes and cosmetic products | [81–83] |
| **BHT** | Increasing the shelf life of animal fats, chewing gum, animal feed, vegetable oils | [82,83] |
| **TBHQ** | As a preservative for enhancing storage stability of vegetable oils, margarine, fish oil, fried foods, essential oils, nuts, edible animal fats, butterfat, and packed fried foods | [82] |
| **Propyl gallate** | As an antioxidant agent in foods and vegetable oil | [82] |

Commercially, synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), α-tocopherol and propyl gallate are used in foods, food packaging, cosmetics and pharmaceutical products [82,84]. However, the physical properties of BHT and BHA (high volatility and instability at elevated temperature), strict
legislation on the use of synthetic food additives, and the carcinogenic nature of some synthetic antioxidants [85–88] have shifted the attention to finding antioxidants from natural sources that are pharmacologically potent and have low or no toxicity.

### 2.4. Generation and Detoxification of ROS

ROS is a collective term for oxygen-derived products (free radicals and non-radicals reactive derivatives of oxygen). They are produced in cellular compartments either exogenously (in response to environmental stress such as UV radiation or xenobiotics) or endogenously (from the intracellular metabolic pathway, enzymatic activities, mitochondrial respiration, or photosynthesis) [66]. Accumulation of ROS leads to oxidative stress in cells and causes damage to cellular macromolecules, including proteins, lipids, carbohydrates and DNA [89]. Various sources of ROS and corresponding modes of biochemical metabolism are summarised in Table 2.

**Table 2. Production of ROS in cells during biological metabolism and their corresponding neutralising antioxidants.**

| ROS Reaction                                                                 | Life Span | Function                                                                                     | Sources of ROS                                                                 | Scavenging Antioxidants |
|------------------------------------------------------------------------------|-----------|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------|
| Chlorophyll triplet state (Chl) is generated due to insufficient energy dissipation during photosynthesis Chl + 3O2 → 1O2 Reduction of transition metal (Fe^{3+}) O2•− + Fe^{3+} → 1O2 + Fe^{2+} | 3 µs (appx.) 4 µs in H2O 100 µs in polar solvents | Gene up-regulation, molecular defense against photo-oxidative stress         | Chloroplast               | β-carotene, lycopene, tocopherol, ASC, plastoquinone, and proline |
| Reduction of oxygen (3O2) during electron transport during the photosynthesis process in chloroplasts or during oxidative phosphorylation in the mitochondria 3O2 + e− → O2•− 3O2 + Xanthine (Xanthine oxidase) → O2•− + uric acid 3O2 + NADPH (NADPH oxidase) → O2•− + NADP+ + H+ | 2–4 µs    | Triggering the formation of more ROS which further participates in membrane lipid peroxidation | PSI in Chloroplast oxidative phosphorylation in mitochondria Peroxisomes Plasma membrane | SOD, ASC, glutathione (GSH), flavonoids, Cu |
| Protonation reaction (acidic conditions) O2•− + H+ + HO2•− → H2O2 + 2O2 Glycolate + O2 (Glycolate oxidase) → H2O2 + Glyoxylate Fatty acids (β-oxidation) → Acetyl coenzyme + H2O2 | 1 ms      | Act as a signaling molecule (low concentration of H2O2), at high concentration of H2O2 triggers tolerance to various stress, as a regulator of physiological processes (photorespiration and photosynthesis) | Chloroplast, Mitochondria, and Peroxisome | CAT, POD (GPX and APX), peroxiredoxin, ASC, tocopherol, GSH, β-carotene, Se flavonoids, lipoic acid |
Table 2. Cont.

| ROS                  | Reaction                                                                 | Life Span | Function                                                  | Sources of ROS                        | Scavenging Antioxidants               |
|----------------------|--------------------------------------------------------------------------|-----------|-----------------------------------------------------------|---------------------------------------|---------------------------------------|
| HO₂•                 | Protonation of superoxide ions                                          |           | Attacks PUFA in the negatively charged membrane surface  | Mitochondria, microsomes and peroxisomes |                                       |
| HO₂• (Three electron reduction of O₂) | Fenton reaction: H₂O₂ + Fe²⁺ → HO²⁺ + HO₂⁻ + Fe³⁺; Haber-Weiss reactions: O₂⁻⁻ + H₂O₂ → HO²⁺ + HO⁻⁻ + O₂ | 1 ps      | Attack unsaturated fatty acids in membranes               | Mitochondria                          | ASc, GSH, flavonoids, lipoic acid, proline |
| Nitric oxide (NO•)   | L-arginine + O₂ → NO• + Citrulline                                        |           | Intercellular messenger, the quencher of O₂•⁻, defense against various protozoa, fungi, and mycobacteria | Peroxisome, cytosol                  | GSH                                   |
| Peroxynitrite (ONOO⁻) | NO• + O₂•⁻ → ONOO⁻                                                       |           | React with amino acids residues in enzymes causing inactivation | Peroxiredoxin, Uric acid            |                                       |
| Lipid hydroperoxide  | Oxidation of PUFA                                                        |           | Tissue injuries and diseases                              | Mitochondrial membrane PUFA         |                                       |

The majority of ROS are generated when electrons leak from the chloroplastic electron transport systems during photosynthesis, from the mitochondrial electron transport chain during photorespiration, and from the peroxisomal membrane electron transport chain (Figure 2) [90]. In plant cells, 1–2% of O₂ consumption leads to the formation of superoxide (O₂•⁻), and 1–5% of mitochondrial O₂ consumption leads to the generation of H₂O₂ [45]. O₂•⁻ is generated during oxidisation of unsaturated fatty acids [91], from the activity of cytochrome P450 [92] and the cytochrome b5 family members [93,94]. Also, O₂•⁻ is produced in the peroxisome where xanthine oxidases catalyse the oxidation of xanthine and hypoxanthine to uric acid [45], and in the plasma membrane due to the reduction of O₂ by NADPH oxidases [49].
Figure 2. Generation of ROS by energy transfer and during intracellular metabolic pathways in microalgae (FD; ferredoxin, FNR; ferredoxin-NADP+ oxidoreductase; PSI-Photosystem I; PSII-Photosystem II, PC-plastocyanin [90,95]).

Superoxide ions (O$_2$•−) are converted into hydrogen peroxide (H$_2$O$_2$) by the catalytic activity of SOD. H$_2$O$_2$ is also produced in peroxisomes when glycolate from the photosynthesis is recycled. In addition, H$_2$O$_2$ can be formed by D-amino acid oxidase, urate oxidase, flavin oxidase, L-α-hydroxy acid oxidase, and fatty acyl-CoA oxidase, and by cell wall peroxidases. Reactive OH• is produced from the reaction of O$_2$•− and H$_2$O$_2$ at neutral pH and ambient temperature (Haber–Weiss reaction) or from H$_2$O$_2$ during the Fenton reaction [96]. In contrast, the addition of a proton (H$^+$) to O$_2$•− generates perhydroxyl radicals (HO$_2$•). In some cases, intracellular ROS could also form during auto-oxidation of small molecules (epinephrine, flavins, and hydroquinones) [97,98]. The singlet oxygen (¹O$_2$) is generated from the reaction of oxygen (³O$_2$) with the triplet state of chlorophyll produced by the dissipation of insufficient energy during photosynthesis [45].

Under normal physiological conditions, ROS is neutralised by the cells’ antioxidant systems where antioxidant enzymes and antioxidant molecules maintain the delicate intracellular redox balance and mitigate undesirable cellular damage caused by ROS, Table 2 [48,50].

Different isozymes of SOD exist (Mn-SOD in mitochondria and peroxisome, Fe-SOD in the chloroplast, and Cu/Zn-SOD isozyme in cytosol) but they all participate in scavenging of O$_2$•−. In addition to SOD, some antioxidants molecules (Vitamin C, glutathione, etc.) also eliminate O$_2$•− [45]. Further, the individual or cumulative catalytic activity of catalase or peroxidases decompose H$_2$O$_2$ into H$_2$O and O$_2$. CAT, peroxidases (GPX, APX) and SOD show a synergistic effect in the scavenging of O$_2$•−. In addition to eliminating H$_2$O$_2$, GPX
can protect cells by preventing intracellular lipid peroxidation [48]. APX may be more efficient compared to CAT or GPX in detoxification of H\textsubscript{2}O\textsubscript{2} due to its higher affinity for H\textsubscript{2}O\textsubscript{2}. APX reduces H\textsubscript{2}O\textsubscript{2} into H\textsubscript{2}O in chloroplasts, cytosol, mitochondria and peroxisomes, and in the apoplastic space using Ascorbic acid (ASC) as an electron donor [99].

Algal cells accumulate ASC with 30–40% remaining in the chloroplast. Ascorbic acid is water-soluble and acts as a potent antioxidant because of its ability to donate electrons in enzymatic and non-enzymatic reactions [100]. It protects cells by directly scavenging O\textsubscript{2}\textsuperscript{•−}, HO\textsubscript{2}\textsuperscript{•−} and regenerating the tocopherol from tocopheroxyl radicals [101]. All intracellular compartments generate the reduced form of glutathione, which plays a role as an excellent scavenger of many ROS such as O\textsubscript{2}\textsuperscript{•−}, HO\textsuperscript{•}, O\textsubscript{3}, NO\textsubscript{2}, lipid hydroperoxides [50] due to the redox-active thiol group that becomes oxidised when GSH reduces ROS [102]. Carotenoid also protects cells from light-induced oxidative stress by quenching \textsuperscript{1}\textsuperscript{O} or dissipating excess heat (excitation energy) or scavenging peroxo radicals [45,48].

As the accumulation of enzymatic and non-enzymatic antioxidants in the cell depends on the external environment, manipulating cultivation conditions could enhance the intracellular antioxidant levels.

3. Cultivation Conditions

In addition to carbon, light energy, and water, Dunaliella requires certain mineral nutrients for growth. A suitable medium can be prepared from natural or artificial seawater enriched with nutrients (carbon, nitrogen, phosphorous, sulphur, iron, and magnesium), trace metals, and vitamins [40]. Carbon dioxide (CO\textsubscript{2}), inorganic carbon (e.g., NaHCO\textsubscript{3}, Na\textsubscript{2}CO\textsubscript{3}), and organic carbon (e.g., sodium acetate, glucose, glycerol) can be used as carbon sources. Nitrate, ammonia, and urea are commonly used as nitrogen sources for the synthesis of amino acids, nucleotides, chlorophylls and phycobilins [103]. Phosphorous is needed for several metabolic processes (ATP, DNA, RNA, and phospholipids). Iron acts as a cofactor for many enzymes (e.g., ferredoxins, catalases, nitrogenases, nitrates), and sulphur is needed for the biosynthesis of specific amino acids (cysteine, methionine). Magnesium is required for chlorophyll biosynthesis, and other trace minerals serve as cofactors for various enzymes. Dunaliella needs light and temperature to assimilate carbon during photosynthesis and enhance biomass productivity and growth [104]. Growth conditions can be classified into three types based on energy and carbon sources (Table 3).

| Characteristics                  | Photo-Autotrophic | Heterotrophic | Mixotrophic                  |
|----------------------------------|-------------------|---------------|------------------------------|
| Carbon assimilation process      | Photosynthesis    | Aerobic respiration | Photosynthesis and aerobic respiration |
| Energy sources                   | Light (solar or artificial) | Organic carbon (e.g., glucose, acetic acid glycerol, wastewaters) | Light and organic carbon |
| Carbon sources                   | Inorganic carbon (CO\textsubscript{2}, NaHCO\textsubscript{3}) | Organic carbon | Inorganic and organic |
| Light energy                     | Mandatory         | Not required  | Not essential                |
| Photo-inhibition effect          | High              | No effect     | Low                          |
| Growth-limiting factor           | Light             | Oxygen        | Light and oxygen             |
| Photo-oxidative damage risk      | High              | Very low      | Protected by accumulating oxygen |
| Types of bioreactors             | Photo-bioreactors and open ponds | Fermenters | Photo-bioreactors, open ponds |
| Availability of bioreactor’s vessels | Photo-bioreactor | Commercially available | Bioreactor |
The essential photoautotrophic growth parameters of microalgae (Dunaliella) are shown in Figure 3.

Photoautotrophic culturing is the most common strategy for growing Dunaliella biomass. However, in large cultures, cell-shading becomes an issue, limiting light penetration into the culture, leading to lower amounts of biomass [107]. In mixotrophic cultivation, the microalgae use CO$_2$ and organic carbon (acetate, glucose) simultaneously along with light energy; respiratory and photosynthetic metabolism operates concurrently [105]. This increases biomass sproduction and results in a higher lipid production compared to that observed in photoautotrophic cultures [107–128], though at increased cultivation costs.

Although heterotrophic cultivation of algae [106] eliminates the requirements for light and facilitates biomass production in the dark, attempts to grow D. para heterotrophically were unsuccessful [110]. However, some Dunaliella strains can grow heterotrophically using organic carbon (such as tryptone, yeast extract, and urea, etc.) as an energy source, together with nutrients [128–133]. No studies have been published supporting the heterotrophic growth of Dunaliella strains at a commercial scale.

Dunaliella can grow phototrophically at temperatures ranging between 10 °C and 30 °C and in a wide variety of media, where the maximum cells density range between 0.3 × 10^6 cells mL$^{-1}$ and 24 × 10^6 cells mL$^{-1}$ [25]. In addition, these species are halotolerant and can grow in a high saline medium (0.5–4.0 M NaCl) [111]. Dunaliella strains can adapt to grow under quite different nutrients conditions, which demonstrates the diversity of these strains [112–115].
Using available technologies, *Dunaliella* biomass production costs are approximately US $4.92 kg\(^{-1}\), which, for biodiesel production, is exceptionally high. Production costs could be reduced (US $3.05 kg\(^{-1}\)) if biomass was produced using waste water as a nutrient source [116]. A biomass production costs of US$ 4.92 kg\(^{-1}\) would be acceptable if biomass was used for the production of high-value products (processed biomass value, US $123 kg\(^{-1}\)) [117]; β-carotene (from *Dunaliella* spp.) and astaxanthin (from *Haematococcus* spp.) command a market value of $100–1000 kg\(^{-1}\) [117]. Moreover, the demand for astaxanthin, β-carotene and lutein is now emerging in the market. The global carotenoid market was valued at €1.3 billion in 2017 and is projected to reach €1.8 billion by 2022 [7]. Other high-value compounds such as antioxidant enzymes could make *Dunaliella* biotechnologies economically profitable. The current biomass productivity of *D. salina* lies between 0.75 g m\(^{-2}\) d\(^{-1}\) and 3.0 g m\(^{-2}\) d\(^{-1}\) (ash-free dry weight, AFDW) [118], depending on seasonality and other cultivation factors.

Variation of growth parameters disturbs intracellular metabolism via photosynthesis and photorespiration, which results in an imbalance between the generation and detoxification of ROS, ultimately stimulating enzymatic and non-enzymatic antioxidant responses. Therefore, modifications of cultivation factors may enhance antioxidants levels, making *Dunaliella* a competitive candidate for antioxidant (enzyme and non-enzyme) production.

4. Tuning Antioxidant Enzyme Activity in *Dunaliella*

There are two ways of inducing stress in *Dunaliella* during growth: biotic and abiotic manipulation [119]. Due to a lack of control over biotic stress, abiotic stress strategies are more sustainable. Abiotic stress conditions are classified as the nutrimental and physical conditions [105,120,121], which are introduced either during the inoculation of cells or during cell growth. Nutrimental factors include:

i. Concentration and source nutrients (e.g., carbon, nitrogen, phosphorus, iron)

ii. Concentration of trace elements (e.g., Zn\(^{2+}\), Cu\(^{2+}\))

iii. Chemicals (e.g., phenol, H\(_2\)O\(_2\))

iv. Heavy metals (e.g., Hg\(^{2+}\), Cd\(^{2+}\))

Physical factors include:

i. Irradiation level

ii. Types of light (direct sunlight, artificial light sources (white, red, blue))

iii. Temperature

iv. pH

v. Salinity

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**Figure 3.** Phototrophic growth parameters essential for *Dunaliella.*
vi. Size of inoculation

4.1. Salinity

Salinity induces osmotic stress, which has been found to affect antioxidant enzyme activity in *Dunaliella* species. Osmotic stress is divided into two types based on the salinity of the medium: hyposaline (salinity < 0.5 M) and hypersaline (salinity > 0.5 M).

CAT and APX activities were found to be highest when the cells were grown at a low salinity level (>0.5 M), with the activity decreasing with increased salinity. In contrast, SOD activity increased twofold at 1 M and threefold at 2 M compared to the activity at 0.5 M NaCl [122]. However, above 2 M NaCl, a decrease in the SOD activity was observed [122]. Above 2 M NaCl, accumulation of glycerol acts as an osmolyte for balancing high salinity-induced osmotic stress [123], which may be the reason for reduced enzymatic antioxidant activity. Similarly, CAT, APX, and GPX activity were found to decrease in *D. tertiolecta* concurrently with an increase in salt concentrations [124], perhaps due to the participation of glutathione (GSH) and ascorbate in the scavenging of H$_2$O$_2$ [124,125]. A higher GR activity in *Dunaliella* species (*salina* and *tertiolecta*) was observed at a low salt concentration (0.1 M NaCl) [124] which would increase the ratio of NADP$^+$/NADPH, thereby ensuring the availability of NADP$^+$ to accept electrons resulting in a reduced flow of electrons to O$_2$ for generation of ROS [126].

On the contrary, a study using *D. tertiolecta* demonstrated that CAT, SOD, DHAR, and GTX activity were unaltered under a wide range (0.05–3.00 M NaCl) of salinities, whilst APX activity increased by 200%, and MDHAR activity by 300% relative to the optimal growth salinity (0.5 M NaCl) [125]. Increased level of enzymatic activity in hypersaline condition may be due to the increase in the formation of ROS (peroxides) or increases in ATP synthesis via the Mehler-peroxidase reaction (which generates ATP by substituting dioxygen for carbon as the electron acceptor) [127]. Also, *Dunaliella* can retain a higher level of Mehler-peroxidase reaction at higher saline conditions [125].

The data above indicates that high salinity-induced osmotic stress is not an effective way to activate all antioxidant enzymes in *Dunaliella* strains. The above investigations also suggest that different *Dunaliella* strains, when exposed to external salinity, show different antioxidant enzyme activity due to differences in resistance and response. Hence, consideration of strain selection must be given if this approach is chosen.

4.2. Light Irradiation

*D. salina* exposed to UV-B irradiation enhanced CAT, SOD, POD activities, reaching maximum levels within three days after they levelled off [128], suggesting that CAT, SOD, POD activity could be part of the cell’s short-term adaptation against UV-B irradiation. Micosporine-like amino acids (shinorine, porphyra, and palythinol) could act as part of a supportive antioxidants network to protect cells against UV-B irradiation or ROS [129]. The synthesis of these compounds could be the reason for the levelling off enzymatic antioxidant response after three days of growth [128]. *D. tertiolecta* exposed to static and fluctuating natural UV radiation (400–700 nm) for short-term (1–3 days) or long-term (4–7 days) showed that only short-term irradiation significantly increased SOD and GR activity compared to non-UV irradiated cells. Glutathione content was reduced under both short and long-term irradiation, whilst APX activity did not significantly change, indicating that natural UV radiation-induced O$_2^-$ was mitigated by SOD, and that H$_2$O$_2$ was detoxified by the consumption of glutathione [130]. In contrast, *D. salina* treated with UV-B irradiation (irradiated cultures for 4 h) and cultured for 15 days increased its CAT, POD, and SOD activity compared to untreated cells [34]. Moreover, APX activity (140 μmol ascorbate mg$^{-1}$ protein h$^{-1}$) was found to increase in *D. bardawil* cells exposed to UV-A radiation as an addition to cultivation under high light energy (150 μmol photons m$^{-2}$ s$^{-1}$) compared to cells cultivated at low light intensities (35 μmol photons m$^{-2}$ s$^{-1}$). Increased APX activation under high light with UV-A radiation may be due to the direct effect of UV-A irradiance on the photosynthetic reactions
that produce increased ROS within the chloroplast and leads to the increased enzymatic activity [131].

Exposing *D. salina* to extremely high light intensities (1000 µmol photons m⁻² s⁻¹) causes excess electron flow in electron transport chains of the photosystem, which leak electrons onto O₂, thereby generating superoxide ion (O₂•⁻) and results in enhancing SOD enzyme activity as the expression level of the antioxidant genes (SOD gene, Fe-SOD) increases [132]. No study has yet shown the effect of high light (>920 µmol photons m⁻² s⁻¹) on CAT activity in *Dunaliella* strains. Perhaps CAT is not involved in mitigating oxidative stress generated during acclimation of *Dunaliella* under high light intensities. Rather, peroxidases or other small antioxidant molecules (carotenoid) have been suggested to participate in the detoxification of ROS, as β-carotene content was found to increase when the algal was cultivated under increasing light intensities [133]. Therefore, CAT, POD, SOD and APX activity in *Dunaliella* can be improved by growing cells under UV radiation combined with a wide range of light irradiation (150–920 µmol photons m⁻² s⁻¹). However, further studies are needed to evaluate the effect of extreme high light-induced (e.g., 1500 µmol photons m⁻² s⁻¹) oxidative stress on antioxidant enzyme response.

### 4.3. Temperature

Temperature affects the microalgal growth rate and the biochemical compositions as the cells’ ability to assimilate nutrients are reduced [134], which can cause intracellular stress.

Cultivation at low temperature (13 °C) together with low light irradiation (20 µmol photons m⁻² s⁻¹) was found to enhance SOD, APX, MDHAR, DHAR activity in *D. salina* compared to the unstressed controls [135], whilst cultivation at a very low temperature (5 °C) decreased CAT activity [136]. Less carotenoid and ascorbate contents was also observed when the cells were cultivated at extremely low temperatures [135]. *D. salina* exposed to low temperature and cultivated under a high light intensity (100–1200 µmol photons m⁻² s⁻¹) was found to increase SOD, MDHAR, GR, APX and POD activity relative to unstressed cells. High light intensities provide saturated CO₂ absorption and the low temperature decreased the rate of CO₂ absorption, which induced photo-oxidative stress and resulted in increased enzymatic activity [35]. SOD, APX, DHAR, activity were increased in *D. salina* (IR-1) when cells were grown for 2 days under high temperature (28 °C) under light irradiation ranging between 100–1200 µmol photons m⁻² s⁻¹ compared to a different strain (Gh-U) [35]. The different oxidative stress responses between two strains could result from variations in resistance to low temperature and high light. Only a few studies describe the effect of temperature on antioxidant enzyme responses in *Dunaliella*, and further investigations are required to explore the effect of temperature on antioxidant enzyme activity.

### 4.4. Nutrients

Deprivation of nitrogen, sulphur, or phosphorous in the culture medium was found to enhance CAT, SOD, and APX activity compared to unstressed *Dunaliella* cells [37,137]. Deprivation of nitrogen reduces chlorophyll content and the synthesis of chloroplastic proteins, whereas deprivation of sulphur decreased the generation of carotenoid and increased ROS levels, which further increased antioxidant enzyme activity. Deprivation of Mn, Zn, Fe was also found to produce an oxidative stress response in *D. salina* with overexpressed SOD isoenzymes [36]. Nitrogen limitation in the growth medium elevated CAT, SOD, and APX levels in *D. salina* [138], indicating that depletion (limitation) of nitrogen also can play a pivotal role as an oxidative stressor for improving antioxidant enzyme activity. Research has also demonstrated that the deprivation of any single nutrient was more effective in enhancing antioxidant enzyme activity relative to combined nutrients deprivation conditions [137].
4.5. Metals

Heavy metals can induce oxidative stress in microalgae, by generating ROS by auto-oxidation, blocking essential functional groups in biomolecules, or substituting essential metal ions [139]. When *D. tertiolecta* was cultured with silver nanoparticle (AgNPs), CAT, SOD, and POD activity increased initially and then decreased over time [140], which indicate that antioxidant enzymes act as a first line of defence to protect the cells by mitigating metal-induced oxidative stress. Nonetheless, antioxidant enzyme activity could not eliminate the metal toxicity during prolonged exposure of cells [140].

Short-term exposur of Hg$^{2+}$ increased APX activity in *D. tertiolecta* compared to un-stressed cells. Hg$^{2+}$ binds to a sulfhydryl group and disturbs the protein functions leading to stress conditions [141]. In contrast, long-term exposur to Hg$^{2+}$ increased β-carotenoid content in *Dunaliella* cells. β-carotenoid acts as a supportive antioxidant. Pre-treatment of *Dunaliella* with Zn followed by cultivation in the presence of ROS generating agents (e.g., H$_2$O$_2$, paraquat) was found to decrease carotenoids level, CAT and APX activities [142], as cells were unable to enhance antioxidants levels. Moreover, the enhanced growth rate indicated that heavy metal toxicity in Zn-treated cells was detoxified by other antioxidants systems (either chelation reactions (metal to PC) or by displacements of Zn from a PC-Zn complex).

Small quantities of Cu$^{2+}$ are essential for growing cells. During cultivation with Cu$^{2+}$ (>5 µM CuCl$_2$) oxidative stress was induced in *Dunaliella* cells [143]. *D. salina* and *D. tertiolecta* exposed to Cu$^{2+}$ in the growth medium had enhanced APX activity. A higher enzymatic activity was measured in *D. tertiolecta* compared to *D. salina*, which could be due to the generation of lower amounts of carotenoid in *D. tertiolecta* [143]. Metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) are also essential micronutrients required for various biochemical and physiological functions during microalgae growth [144]. However, non-essential heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are systematic toxicants [145]. Epidemiological and experimental studies have shown that these metals have adverse effects on health in humans, including cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, diabetes, hearing loss, hematologic and immunologic disorders, and various types of cancer, an association between exposure and cancer incidence in humans and animals [146]. Using heavy metals enriched biomass in the food industry or as a feed in the aquaculture industry is not a viable strategy to induce stress in algae cultures.

4.6. Chemicals

Chemicals modulate cellular metabolism and produce oxidative stress resulting in higher antioxidant levels in cells [147]. Culturing *D. bardawil* with a mixture of two surfactants (sodium dodecylbenzene sulfonate (SDBS) and cetyl trimethyl ammonium chloride (CTAC)) induced oxidative stress, increasing CAT and SOD activity compared to untreated cells [148].

The exposure of *D. salina* to 2-chlorophenol increased CAT, SOD, and GST activities to mitigate oxidative stress, which was produced by protein carbonyls formation through metal catalyzed oxidation reaction [149]. However, APX activity decreased when *D. salina* was cultured with 2-chlorophenol. Increased level of GST activity may be due to the ability of GST to catalyze the reduced glutathione in the presence of phenol [149]. Moreover, GST can also reduce lipid hydroperoxides to the corresponding alcohols [150]. Investigation of the effect of two pesticides on the antioxidant response in the culture of *Dunaliella* demonstrated that both pollutants (trichlorfon and dimehypo) at a low concentration induced oxidative stress that stimulated the activity of CAT compared to untreated cells [151]. CAT activity in *D. salina* was improved when cells were grown with herbicides (methylene blue and norflurazon) under low-temperature conditions [152]. Herbicides suppressed the synthesis of chlorophyll and decreased the carotenoid biosynthesis leading to an increase
in CAT activity. Therefore, phenolic compounds or herbicides as stressors during Dunaliella culturing could be an effective strategy for enhancing enzyme activity.

4.7. Combination of Abiotic Factors on Antioxidant Enzyme Activity

Most research on antioxidant enzyme activity in Dunaliella focuses on only one factor, with a few studies assessing the combined effect of two factors. The studies indicate that a combination of factors is more effective in producing enzymatic antioxidant responses in Dunaliella compared to exposure to a single stressor [37,103,137]. Factors that can be combined to produce an antioxidant response include UV-B radiation + high salinity + nitrogen deficiency (enhanced CAT, SOD, POD activities).

UV-B radiation combined with high salinity and nitrogen deficiency enhanced CAT, SOD, POD activity relative to the non-radiated cells grown under normal growth conditions [34]. The combined effect of UV-B irradiation and nitrogen starvation can produce oxidative stress, which was eliminated by enhanced SOD, APX, and GPX activities relative to untreated D. salina cells, whilst GR activity did not significantly change. However, the combined effect on antioxidant enzyme activity was significantly lower relative to the effect of either UV-B radiation or nitrogen deprivation [37]. Therefore, the combination of UV-B irradiation and nitrogen deprivation may not be effective in enhancing antioxidant enzyme activity in Dunaliella. Salinity (2–3 M NaCl) associated with propyl gallate can enhance CAT and APX activity, whilst SOD activity was unaltered in D. salina compared to unstressed cells [152] suggests that salinity associated with other chemical-induced stress may increase in antioxidant enzyme activity in Dunaliella. There is a need for further research into this are to develop a deeper understanding of the ‘tuning parameters’ for enhanced enzymatic activity. The effects of abiotic factors on antioxidant enzyme activity are summarised in Table 4.

Table 4. Antioxidant enzymatic response of Dunaliella exposed to different abiotic exogenous stress.

| Stress Conditions | Strains | Optimum Light (µmol Photon m⁻² s⁻¹) | Salinity (NaCl) (M) | Temperature (°C) | Antioxidant Enzymatic Response | References |
|-------------------|---------|--------------------------------------|---------------------|------------------|------------------------------|------------|
| Salinity          |         |                                      |                     |                  |                              |            |
| 0.05–3.00 M NaCl  | D. tertiolecta (UTEX999) | 150 Continuous light | 0.1–0.5 | 26   | No effect on SOD and CAT, GTR activities, APX increased (2-fold) at high salinities (0.2–3 M) | [125]       |
| 0.5–5.5 M NaCl    | D. salina | 150 Light: dark cycle (12:12 h) | 1.5 | (25 ± 2) | CAT activity decreases with salinity, SOD increased (0.5–2 M) and then decrease APX decreases (0.5–2 M) and then constant CAT and SOD activities increase at hypo saline condition in D. tertiolecta | [122]       |
| 0.05–4.0 M NaCl   | D. salina and D. tertiolecta | 150 Light: dark cycle (12:12 h) | 1.25 | (25 ± 2) | APX activity in D. tertiolecta higher at hypo saline and lower at hyper saline condition, GPX activity decreased in D. tertiolecta at higher saline condition (>1.25 M) | [124]       |
Table 4. Cont.

| Stress Conditions | Strains         | Optimum Light (µmol Photon m⁻² s⁻¹) | Salinity (NaCl) (M) | Temperature (°C) | Antioxidant Enzymatic Response | References |
|-------------------|----------------|-------------------------------------|---------------------|-----------------|--------------------------------|------------|
| Light             | D. salina      | 54 (Continuous)                     | 2                   | 22              | APX increase (171%) at 4 M     | [153]      |
| Light             | D. tertiolecta | Light: dark cycle (12:12 h)         | -                   | 20              | SOD activity slightly changed  | [130]      |
|                   | D. salina      | Light: dark cycle (12:12 h)         | -                   | 20              | but no change in APX and GTX   |            |
|                   | D. tertiolecta | 250                                 | -                   | 20              | activity increased on 3rd day   |            |
|                   | D. salina      | 60–80 Light: dark cycle (12:12 h)   | -                   | 20              | SOD activity increased on 4th   | [128]      |
|                   | D. bardawil    | UV-A                                | -                   | 20              | day POD activity increased on   |            |
|                   | D. salina      | 150                                 | -                   | 25              | 3rd day                         |            |
|                   | D. salina      | 150 Continuous light                | -                   | 30              | SOD and CAT activity increased  |            |
|                   | D. salina      | 60 (Continuous)                     | -                   | 25              | CAT, SOD and POD activity       |            |
|                   | D. salina      | 150 Continuous light                | -                   | 25              | increased with increased        |            |
|                   | D. salina      | 70 Light: dark cycle (16:8 h)       | 20                  | 20              | MDS overexpressed               | [36]       |
|                   | D. salina      | 85 Continuous light                 | 25 ±5               | -               | POD and APX activity increased  | [37]       |
|                   | D. salina      | 100 Light: dark (16:8 h)            | 28 ± 0.5            | -               | Mn-SOD overexpressed            | [36]       |
|                   | D. salina      | 100 Light: dark (16:8 h)            | 28 ± 0.5            | -               | SOD activity increased          | [35]       |
|                   | D. salina      | 100 Light: dark (16:8 h)            | 28 ± 0.5            | -               | Mn-SOD overexpressed            | [36]       |
|                   | D. salina      | 54 Continuous light                 | 22                  | 54              | APX activity increased          | [145]      |
|                   | D. tertiolecta | (Continuous light)                  | 25                  | 54              | APX activity increased          | [141]      |
|                   | D. salina      | (Continuous light)                  | 25                  | 54              | APX activity increased          | [141]      |
Table 4. Cont.

| Stress Conditions | Strains | Optimum Light (umol Photon m⁻² s⁻¹) | Salinity (NaCl) (M) | Temperature (°C) | Antioxidant Enzymatic Response | References |
|-------------------|---------|-----------------------------------|-------------------|-----------------|-------------------------------|------------|
| Cd²⁺ (0–20 µg L⁻¹) for 24, 48,72, and 96 h | *D. viridis* | 117 (Light: dark) (12:12 h) | (28 ± 2) | Pigment production decreased | [154] |
| Cr⁶⁺ (40 ppm) for 24 h | *D. salina and D. tertiolecta* | 46 White fluorescent light | 25 | SOD activity decreased Chlorophyll ‘a’ content decreased with increase in the exposure time CAT activity increased until 6 days then decreased, SOD activity increased up to 4 days, POD activity increased until 6 days | [155] |
| Silver nanoparticles (10, 50, 100 and 200 mg L⁻¹ Ag-NPs) for 8 days | *(D. tertiolecta)* | 100 Continuous light | 25 | | [140] |
| Chemicals | *D. tertiolecta* (CCAP19/6B) | 58 Continuous | (24 ± 1) | Increased carotenoid | [156] |
| Carbamazepine (CBZ) (0–200 mg L⁻¹) for 24, 48,72, and 96 h | *D. bardawil* | 144 (Light: dark) (14:10 h) | 26 | CAT and SOD activity increased | [148] |
| SDBS and CTAC for 48 h | *D. salina* | 54 (Light: dark) (14:10 h) | 26 | CAT activity increased | [151] |
| Trichlorfon and dimehypo 2-chlorophenol for 24, 48, and 96 h | *D. salina* | 100 (Continuous daylight) | (25 ± 1 °C) | SOD, CAT, GTX activity increased APX activity decreased | [149] |
| Chemical and salinity | *D. salina* (UTEX 200) | 70 Light: dark (16:8 h) | 25 | Maximum CAT activity at 2 M NaCl and no change in SOD activity at 1 and 3 M NaCl Minor increase in APX activity at 3 M NaCl CAT activity increased Chlorophyll and carotenoid content decreased with temperature | [152] |
| Chemical and temperature | *D. salina* (IPASS D-294) | 73.6 White fluorescent light | 27 | | [136] |

### 5. Culturing Systems

Commercially, large-scale microalgae biomass is produced in open ponds, raceway ponds, enclosed photobioreactors (PBRs), or a combination of hybrid systems [157]. The cultivation of *Dunaliella* also employs these various production systems [40,106,158–161] depending on the location and the desired end-products.

The PBR is considered a suitable production method for microalgae (*Dunaliella*) cultivation to enhance antioxidant production with biomass productivity in a ‘monoculture’ and to maintain culturing reproducibility. This method is flexible, controllable, and able to provide sustainable biomass production [105,162]. In open pond systems, the cell concentration is lower compared to that obtained during PBR cultivation as external factors (light irradiation, temperature, evaporation, contamination, aeration) are difficult to control. However, PBRs are not yet outperforming open pond systems in production volume and costs [40]. To the best of knowledge, no studies so far have reported the use of specific PBRs to culture *Dunaliella* strains to enhance enzymatic antioxidants and non-enzymatic
antioxidants. The effect of the production system on antioxidants needs to be studied further.

6. Post-Culture Downstream Processing

High-cost downstream processing is one of the major challenges to making microalgae bio-refinery economically profitable [10,163]. Technological developments in harvesting, post-harvest storage, cell disruption, extraction, and possibly fractionation are needed. Harvesting the cells from the growth medium at the end of the culturing process is one of the most critical and challenging steps in mass Dunaliella culture [164].

6.1. Harvesting

The selection of appropriate harvesting techniques is dependent on the characteristics of the microalgae. Considerable challenges in harvesting Dunaliella cells are the lack of a rigid cell wall, its small size (length 5–25 µm), the low cell density (0.1%) in the culture medium, and the high salinity (2–3 M NaCl) of growth medium. Traditionally, harvesting procedures to concentrate microalgae cells are filtration, sedimentation, flocculation, flotation, centrifugation [163–165]. Several authors have extensively reviewed the harvesting of microalgae, and in the following section, a few key points are made with particular reference to Dunaliella [164,166–168].

6.2. Post-Harvesting Storage Processing

For Dunaliella to be a viable and sustainable natural antioxidant enzyme source, suitable drying and storage of biomass must be established. Optimized post-harvest storage conditions can minimise the loss of antioxidant enzyme activity and increase these valuable compounds’ shelf life. Cold storage is commonly used to preserve chemical, nutritional, and sensory properties of post-harvest processed living cells as respiration and other metabolic reactions are reduced at lower temperatures [169]. Biomass can be prepared for cold storage via a dry or a wet route. In the dry route, Dunaliella biomass is freeze-dried or air-dried, then stored at cold temperature. In contrast, in a wet route, biomass is preserved at cold temperature straightway [25]. Several reports demonstrate that antioxidant stability depends on the type of biomass, type of chemical components, length of storage time, storage temperature, and drying method [170–173]. Roy et al., (2020) compared the storages of both wet and freeze-dried algal biomass and suggested that CAT and SOD activity in freeze-dried D. tertiolecta biomass can be retained for eight months when stored at −20 °C, whereas activities in wet biomass or crude extract remain unchanged for four months when stored at −80 °C [173–175]. Therefore, using wet biomass, either fresh or frozen, as feedstock may be an economically feasible method for antioxidant enzyme storage compared to dried cell storage. Extraction procedure of antioxidants from algae biomass depends on the desired products. For example, lipid-soluble antioxidants (e.g., carotenoids, tocopherol, flavonoids, ascorbate) are extracted by organic solvents (e.g., hexane, heptane, methyl tertiary buterate, methanol, ethanol) with a mechanical treatment (e.g., ultrasonication, microwave, high pressure) [2,10,16,174]. Water-soluble antioxidants or antioxidant enzymes are recovered from the algae biomass by aqueous solvent or buffer systems together with mechanical assistance [37,128,138,175,176]. Recently, green solvents (e.g., cyclopentyl methyl ether (CPME), dimethyl carbonate (DMC)), ionic liquids (e.g., ethanol containing 1-n-butyl-3-methylimidazolium) and supercritical CO2—based extraction have been used for the recovery of carotenoids (e.g., β-carotene, astaxanthin) [7,177].

7. Challenges and Future Opportunities

Despite the potential of Dunaliella as a source for high-value compounds including antioxidants, the main challenges are as follows:

i. Low growth rate and productivity of Dunaliella strain [4,28,174] relative to other sources (such as fungus, yeast) [178–181] and selection of a suitable strain;

ii. Transferring lab-scale optimised abiotic stress strategies to a commercial scale;
iii. Seasonal and environmental variation in biomass production, and the control of competitors in open ponds cultivation systems;
iv. Engineering developments of sustainable large-scale technologies (open raceways ponds and PBR) for Dunaliella and microalgae in general for efficient biomass and enzymatic antioxidant production;
v. Developing sustainable downstream technologies (harvesting, extraction, and purification) for antioxidant enzymes production at commercial scale;
vi. Establishing tools for proteomics, genomics, and metabolomics, and implement them to develop strains for the accumulation of intracellular antioxidant enzymes;
vii. Stabilisation of antioxidant enzymes in food ingredients and extension of their shelf life;
viii. Algae extracts can be used as taste enhances in foods; however, some strains are associated with a bitter or astringent taste;
ix. Introducing a general platform to solve regulatory and labelling issues, as this varies from country to country.

Based on the reviewed literature, emphasis has been on carotenoid production from Dunaliella, limiting the exploration of Dunaliella biomass to produce other high-value compounds such as antioxidant enzymes. The tolerance of Dunaliella to extreme environments (such as high salinity and high light levels) makes these strains versatile. It enables them to survive in stressful environments, providing opportunities to explore these strains as natural antioxidant enzyme sources either as a main product or by-products. In addition, there is an opportunity to improve technological developments in harvesting biomass by employing cost-effective processes (such as flocculation or microbubbles techniques). Due to the lack of a rigid cell wall, the extraction process is cheaper relative to other algae cells or plants, which will reduce downstream processing costs. As antioxidant enzymes in wet Dunaliella biomass can be retained for one month when stored at $-20 \, ^\circ C$ [175]. The development of an extraction procedure for antioxidant enzymes from wet biomass can save the energy costs required for drying the biomass.

Techno-economic assessment can be conducted to assure the profitability of developed cultivation and downstream processing systems to produce specific antioxidants enzymes or biorefinery strategies. There is also an opportunity to explore novel applications of Dunaliella-derived antioxidant enzymes in the pharmaceuticals, nutraceuticals, agricultural and cosmetic industries. Growing end-user applications for antioxidant enzymes and increasing end-user awareness may enhance the demand in the global market.

8. Conclusions

Dunaliella can accumulate a wide range of molecules, with a high value in the global food, aquaculture, animal feed, pharmaceutical, and nutraceutical industry. Currently, β-carotene production from D. salina is commercially cost-effective. High biomass production costs; lack of optimal cultivation conditions and culturing systems; technical difficulties in scaling-up; lack of cost-effective downstream processing technologies; and instability of antioxidant enzymes at room temperature are the major constraints to the commercialisation of Dunaliella-based antioxidant enzyme production. Tuning abiotic stress factors during cultivation can activate different antioxidant systems in Dunaliella. A multiple factors-based stress strategy is more effective relative to a single inducing stress factor during growth in a photobioreactor (considered monoculture) system, giving design flexibility and controllability.

Author Contributions: Conceptualisation U.K.R., B.V.N., J.J.M.; Writing—original manuscript preparation U.K.R.; Writing—review and editing U.K.R., B.V.N. and J.J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

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