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Enhancing algal production strategies: strain selection, AI-informed cultivation, and mutagenesis

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Microalgae are emerging as a sustainable source of bioproducts, including food, animal feed, nutraceuticals, and biofuels. This review emphasizes the need to carefully select suitable species and highlights the importance of strain optimization to enhance the feasibility of developing algae as a sustainable resource for food and biomaterial production. It discusses microalgal bioprospecting methods, different types of cultivation systems, microalgal biomass yields, and cultivation using wastewater. The paper highlights advances in artificial intelligence that can optimize algal productivity and overcome the limitations faced in current microalgal industries. Additionally, the potential of UV mutagenesis combined with high-throughput screening is examined as a strategy for generating improved strains without introducing foreign genetic material. The necessity of a multifaceted optimization approach for enhanced productivity is acknowledged. This review provides an overview of recent developments crucial for the commercial success of microalgal production.

KEYWORDS

microalgae, bioprospecting, ultraviolet (UV) mutagenesis, strain selection, artificial intelligence

1 Introduction

Finding alternative sources to fulfill food and fuel security needs is imperative, especially in impoverished regions or those heavily relying on imports. For example, Middle Eastern and North African countries, dependent on food imports, were significantly affected during the COVID-19 pandemic. These regions are also predicted to face accelerated hardships, especially in regard to their food and fuel supplies, due to global conflicts (Ben Hassen and El Bilali, 2022). In a broader sense, alternative food and fuel sources are needed for the ever-growing human population, either to supplant or complement current resources, to prevent resource scarcity in the future.

Microalgae and their high-value bioproducts have the potential to address food and fuel security issues due to their advantages in commercial production. Microalgae are unicellular photosynthetic microbes that have been widely studied and used for the sustainable production of vitamins and carotenoids (Abo et al., 2019; Fu et al., 2019). They can grow photoautotrophically using carbon dioxide and minimal salts media or

heterotrophically using a wide range of organic compounds as an energy source (Carino and Vital, 2022). Their faster growth rate, higher biomass productivity, lack of competition with crops for arable land and freshwater (Diaz et al., 2023), and capacity for sustainable growth using marginal resources make microalgae more favorable organisms than higher plants for mass production where land and water are limiting resources (Abo et al., 2019; Fu et al., 2019).

Microalgae produce up to 31 times higher lipid yield per hectare in comparison to other oil-producing crops (Udayan et al., 2022). They have a suitable size for ingestion, and their cell walls are highly digestible (Abo et al., 2019). These organisms also produce nutrients found in higher plants, such as proteins, nucleic acids, carbohydrates, fibers, starches, vitamins, antioxidants, and polyunsaturated fatty acids (Jareonsin and Pumas, 2021). Some microalgae species are promising for biodiesel production due to the high lipid content of 50–80% of their biomass (Khan et al., 2018). This sustainable energy source is eco-friendly, non-toxic, and presents a strong potential for CO_2 reduction. Utilizing microalgae biomass to manufacture various biofuels adds new inputs to the CO_2 sequestration process, which helps maintain a stable atmospheric CO_2 level and a clean, safe environment (Jaiswal et al., 2022). Many countries across Asia, Europe, and America have started the industrialization of bioenergy derived from microalgae biomass (Khan et al., 2018). For instance, in 2019, America produced 1,551.1 peta joules (PJ) of biofuel, followed by Brazil with 992.2 PJ and Europe with 403.3 PJ (Khoo et al., 2023). The global market for microalgae-based products is estimated to increase from $32.60 billion in 2017 to $53.43 billion in 2026 (Rahman, 2020; Rafa et al., 2021). Governments around the globe aim to integrate biofuels into their transport fuel sectors, with the estimation that biofuels will constitute 20% of liquid fuels by 2050 (Khoo et al., 2023). However, despite these initiatives and clear advantages over land crops, the current state of the microalgal industry is insufficient to address wider issues in food and fuel security.

Advancements in microalgal technology focus on reducing production costs (Xie et al., 2022), as the processing stages of cultivation and harvesting are currently too costly to produce adequate profit margins (Khoo et al., 2023). Such technologies can be biological, for example, by the overexpression of carbon anhydrase (CA) to improve microalgae photosynthesis efficiency or through gene manipulation of fatty acid acyl thioesterase and acyl-CoA synthase (ACS) to produce extracellular fatty acids; additional approaches include producing fatty acid ethyl esters (biodiesel) through pyruvate decarboxylase, alcohol dehydrogenase and wax ester synthase, or through stearoyl-ACPΔ9 saturase, thioesterase, ACS and desaturase to produce high-quality biodiesel (Xie et al., 2022). These modifications ensure that growth and lipid productivity in the chosen microalgal strains are simultaneously optimized (Khoo et al., 2023). Subjecting microalgae to high salinity and nitrogen deprivation leads to overproducing lipids due to the algae's attempt to establish an equilibrium within the system (Russell et al., 2022). Nutrient starvation is a notable strategy to amplify lipid content in microalgae; although this often compromises biomass yield (Kim et al., 2023), new approaches for strain optimization can overcome this hurdle. Wider dissemination of targeted information, such as presented in this review, can assist strain selection and optimization efforts that can in turn alleviate high costs associated with genetic alterations limits imposed by proprietary technologies (Varela Villarreal et al., 2020). Inducing mutagenesis by physical methods such as UV rays has been an efficient technique for strain improvement and increased lipids in microalgae (Sivaramakrishnan and Incharoensakdi, 2023). Since this method is scalable and inexpensive, it may be the best strain optimization technique for broad application to improve microalgae production strains.

This review outlines several important steps to improve microalgal productivity to fulfill food and fuel security needs at a larger scale. It describes important steps for generating production-level strains, including bioprospecting and strain optimization. Specifically, the application of UV mutagenesis combined with a precise selection technique to successfully enhance microalgal lipids without affecting biomass productivity is highlighted.

2 Microalgal bioprospecting: site selection, isolation, and decontamination techniques

Microalgal bioprospecting is an essential first step to working with a new biotechnology candidate strain. The inception involves collecting samples from diverse aquatic and terrestrial habitats. Freshwater, terrestrial, and marine environments, including soil (Leitner et al., 2022), lakes (Berges et al., 2021), rivers (Kumar et al., 2023), and coastal areas (Nelson et al., 2017), serve as rich sources for novel microalgal isolates; although, microalgae can be isolated from harsh environments including desert sand (Nelson et al., 2019), cryospheric (Nelson et al., 2013) or lithospheric (Häubner et al., 2006) surfaces, as shown in Table 1. In sample collection, exemplified in Figure 1, the documentation of each collection site's geographical coordinates, temperature, pH, and salinity provides essential context for downstream studies.

Algal lipids are highly diverse bioproducts whose accumulation and characteristics vary based on the strain's ancestry and environment. These differences in lipid types help maintain cell membrane stability under different conditions and represent a wide variety of bioprospecting opportunities. Species that can withstand cold temperatures, like those from polar, boreal, and alpine areas, tend to have abundant long-chain polyunsaturated acids. For example, the nutraceutical, very-long-chain fatty acids eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) are more likely to be found in abundance in alpine (Nelson et al., 2013), polar, and cold temperate regions (Boelen et al., 2013). Species from warmer climates usually have shorter fatty acids. For example, we recently reported isolating high palmitic acid (C16:0) strains from desert regions. The green alga *Chloroidium* sp. was isolated from multiple locations in the United Arab Emirates (UAE) in a screen for lipid-producing algae (Sharma et al., 2015). We found that 41.8% of its total fatty acids consisted of palmitic acid (Nelson et al., 2017), a value close to the 44% palmitic acid found in the palm tree *Elaeis guineensis* (Mancini et al., 2015). The nearly equivalent lipid profiles in oil palm and the desert alga

TABLE 1 Microalgal bioproduct accumulation varies with the strain's ancestry and environment.

Environment	Species	Molecules of interest	Enhancement	References
Marine, coastal	*Heamatococcus pluvialis, Chlorococcum, Chlorella zofingiensis*	Terpenoids, furanones, astaxanthin	Yes—Chemical mutagesis for cell wall mutants, transformation, omics data	Wang et al., 2005; Darwesh et al., 2022; Zhang et al., 2023
Alpine	*Heterococcus sp.*	EPA, DHA	No	Nelson et al., 2013
Coastal, subtropical	*Chloroidium sp. 3007*	Palmitic acid	Yes—UV mutagenesis for palmitic acid increase, omics data	Nelson et al., 2017
Benthic marine	*Cylindrotheca closterium*	Anti-inflammatory	No	Elagoz et al., 2020; Wan Afifudeen et al., 2022
Salt flats, desert	*Dunaliella tertiolecta*	Myricetin	Yes, chemical lipid production increase	Lopez et al., 2015; Chen et al., 2019
Marine, coastal	*Schizochytrium*	DHA, squalene	Yes—transformation, omics data	Liu et al., 2022; Wang and Zhang, 2022; Ahn et al., 2023; Helmy et al., 2023
Marine, coastal	*Tisochrysis lutea, Heterocapsa sp.*	Exopolysaccharides, xanthophylls	Yes, omics data	Mohamadnia et al., 2022; Concordio-Reis et al., 2023; Lacour et al., 2023
Marine	*Nannochloropsis* sp.	DHA, EPA	Yes—transformation, omics data	Gong et al., 2023; Helmy et al., 2023

Listed are examples of strain isolation environments, produced molecules of interest, and example enhancement strategies.

revealed the alga as a promising candidate for sustainable palm oil alternatives.

Microalgal strains are extracted from environmental samples using specialized microbiology techniques. Isolation techniques include direct plating, serial dilution, and single-cell isolation. Single-cell isolation can be done with microscopy, fluorescence-activated cell sorting (FACS), and generating aerosol clouds where individual droplets contain one cell of the target species (Nelson et al., 2013). In microscopy-based isolation, cells are observed under a microscope, and individual cells are picked using a micropipette (Lue et al., 2022). Sampled cells can be plated on solid media or inoculated into liquid media. These meticulous methods ensure the isolation of pure strains, removing possible xenic confounding factors from downstream projects. Several routes for the early culturing of isolates are available. Water or media-based samples are spread onto agar plates containing minimal salts medium (e.g., F/2; Lananan et al., 2013). Incubation conditions typically involve a 16:8 light-dark cycle at 25°C for several weeks until colonies emerge and can be picked for transfer.

Algal strains usually need to grow axenically for proper characterization and to make high-value products. Decontamination procedures include antibiotic treatments, repeated sub-culturing, and physical separation (Andersen, 2004). These methods ensure the purity of isolated strains for subsequent applications and research. To eliminate bacterial contaminants, cultures can be treated with antibiotics, commonly penicillin and streptomycin (50–100 μg/mL). This is most commonly done by adding antibiotics to solid media in agar-based Petri dishes, but antibiotics added to liquid media, such as in the serial dilution technique, are also effective. Monitoring bacterial growth is essential, and treatments may be repeated as necessary. The serial dilution technique is beneficial for samples with high microbial density or recalcitrance to solid media culture. Serial transfer involves transferring a portion of the culture to fresh media multiple times. It aids in gradually eliminating contaminants, ensuring a pure microalgal culture. Certain kinds of persistent contamination may require density gradient centrifugation (Okumura et al., 2015). Here, cells are separated from contaminants based on density, although maintaining cell viability is challenging in this technique.

Post-isolation, strains are identified and evaluated for commercial potential based on morphological characteristics using microscopy techniques (Deniset-Besseau et al., 2021; Sonmez et al., 2023). Identification through DNA barcoding, mainly 18S rRNA gene sequencing, is a precise and widely used method for strain determination (Darienko et al., 2015; Hadi et al., 2016; Zou et al., 2016). After identification, candidate bioactive compounds are extracted and subjected to various assays to ascertain their bioactivity (Uzlasir et al., 2023; Yang et al., 2023; Zhou et al., 2023). Recent bioprospecting endeavors have unveiled a plethora of microalgal strains with promising bioactivities. For instance, certain strains have demonstrated antioxidant (Yang et al., 2023), antimicrobial (Zhou et al., 2023), and anti-inflammatory properties (Leitner et al., 2022; Kurniawan et al., 2023; Manabe et al., 2023). Moreover, some strains are suited for biofuel production due to their high lipid content (Nelson et al., 2013, 2017; Deniset-Besseau et al., 2021). Microalgal bioprospecting stands at the forefront of discovering novel compounds with diverse applications. The techniques employed, from isolation to decontamination, play a pivotal role in ensuring the success of these endeavors. As research in this domain intensifies, refining these techniques and adopting innovative approaches will be instrumental in harnessing the full potential of microalgae.

Research areas for future studies on microalgal bioprospecting from diverse environments include increasing studies in Asia and Africa. For example, the exceptionally high marine biodiversity region of the Coral Triangle (CT; Tornabene et al., 2015; Williams et al., 2017; Ampou et al., 2018) consisting of the tropical waters

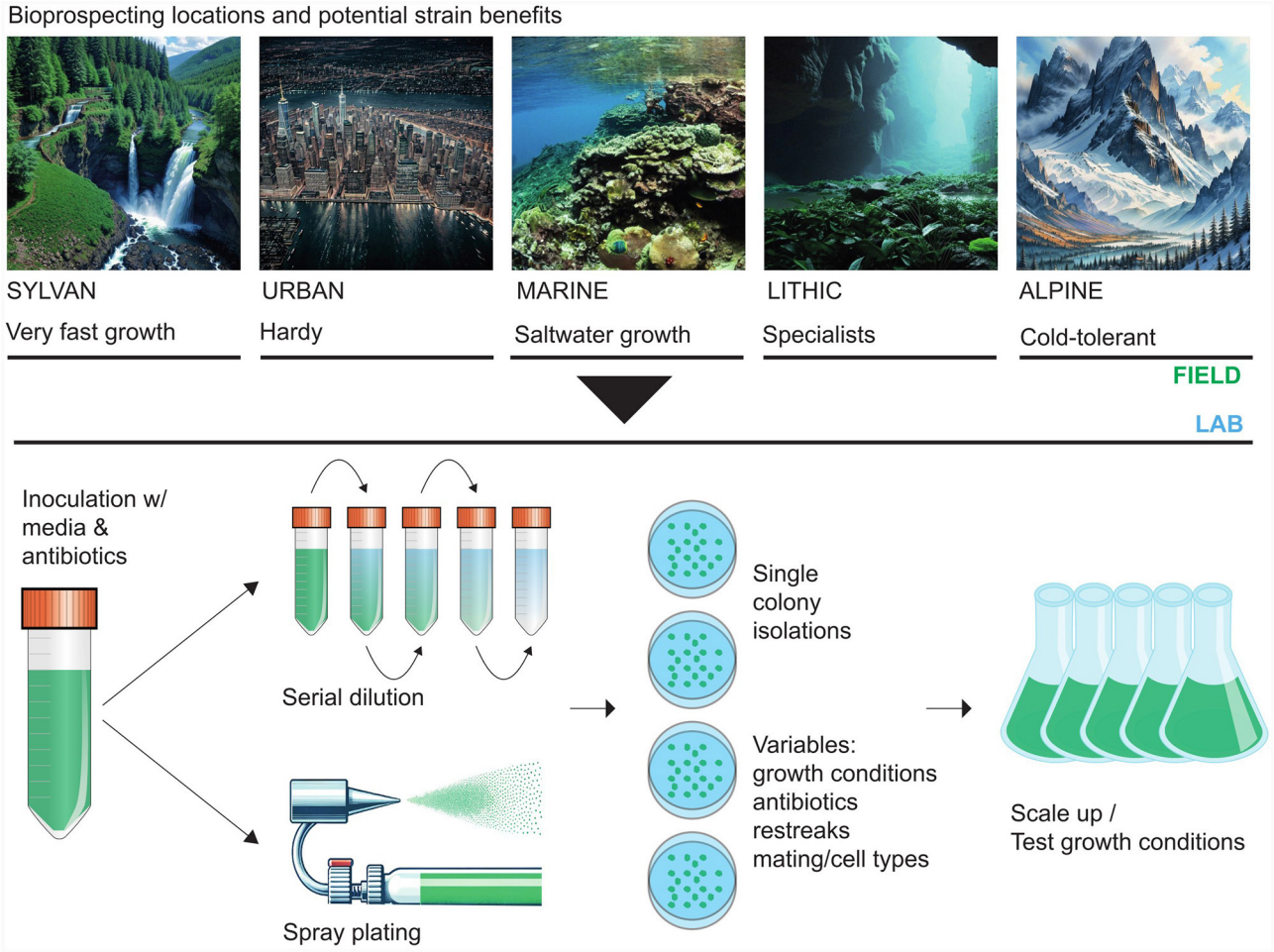

FIGURE 1
Field locations and laboratory methods for microalgal bioprospecting. Microalgae occur in a wide range of environments. Adaptations to these varying conditions can manifest the production of different metabolites suitable for different applications (top panel). After sampling, single colony (isogenic) and decontaminated (axenic) cultures can be obtained through several physical and chemical protocols. Once a suitable, isogenic, axenic strain has been obtained, downstream analyses and scale-up operations can be carried out (bottom panel). The search for algae that produce specific compounds of interest can be narrowed by considering that these compounds are generally environmentally-responsive. Abiotic or biotic factors can cajole an algal species into making commercially profitable bioproducts. For example, harmful algal blooms, which produce various toxins, can be a rich source of potent pharmaceuticals (Pradhan and Ki, 2022). Ultraviolet light also promotes the accumulation of some useful bioproducts. For example, the high-value pigment astaxanthin is more likely to accumulate in strains subjected to intense UV irradiation in their natural environment, and the production of astaxanthin increases with higher UV levels (Wu et al., 2010).

by the Philippines, Malaysia, Papua New Guinea, Timor-Leste, the Solomon Islands, and Indonesia, would be a very rich source of algal isolates, as would each of the remote islands in that area of the Pacific Ocean. Intensifying bioprospecting efforts in less-studied regions will likely lead to valuable discoveries of new strains with commercial potential.

Most known algal isolates are held in culture centers, predominantly in the Americas and Europe. For example, the Culture Collection of Algae at the University of Texas (UTEX, https://utex.org/), the Scottish Association for Marine Science (SAMS, https://www.sams.ac.uk/), the Culture Collection of Cryogenic Algae (CCCryo, http://cccryo.fraunhofer.de/), and the Bigelow National Center for Marine Algae and Microbiota (NCMA, https://ncma.bigelow.org/) are a few prominent examples of large algal collections. Isolates from these collections are available with few restrictions to the greater scientific community and can be explored for their suitability for various applications.

3 Microalgae cultivation methods

Cultivating microalgae using various methods for commercial (Araújo et al., 2021) and economical high-value products requires mass cultivation, and it should be economically feasible (Shekh et al., 2022). It is estimated that algal biomass production costs about 11 USD per 1 kg in 1 acre of area, which is high as compared to biodiesel obtained from other plants (Khoo et al., 2023); therefore, it is desirable first to assess the suitability of a strain for mass cultivation in the laboratory setting.

Lab-scale culture systems that simulate light and temperature settings can test a strain's biomass productivity in outdoor ponds,

speeding up strain characterization and lowering the costs, time, and effort involved in setting up and running ponds (Huesemann et al., 2017). Pond simulators such as the indoor climate simulation raceway developed by Pacific Northwest National Laboratory (PNNL), and the benchtop environmental photobioreactor (ePBR) manufactured by Phenometrics, Inc. (Lansing, MI, USA) are capable of mimicking the growth behavior of microalgae cultured in outdoor ponds to faithfully reproduce the microalgal growth performance of outdoor cultures exposed to varying levels of light intensity and water temperature (Huesemann et al., 2017). A study compared the biomass productivities of *Chlorella sorokiniana* DOE 1412 in PNNL climate-simulation ponds and outdoor ponds, showing no statistically significant difference (Huesemann et al., 2017; Skeffington and Scheffel, 2018).

3.1 Open and closed cultivation systems

The main cultivation systems employed in large-scale commercial biomass production are open and closed culture configurations that are used to obtain different chemical compounds, such as fatty acids, proteins, antioxidants, pigments, and animal feedstock (Alishah Aratboni et al., 2019). Table 2 presents a detailed comparison of both cultivation systems, highlighting the advantages and disadvantages of each system. These two cultivation systems support both suspension and attached cultures. However, attached microalgae cultivation for biofuel production has been more favorable than suspended cultivation, as shown in Table 3, because it requires less space, has a low process cost, and is simple to harvest cultivated culture.

The commercial cultivation of *Arthrospira* in open raceways or tubular photobioreactors (PBRs) in over twenty countries, and *Chlorella* in open ponds, cascades, enclosed tubular and circular bonds in Japan, China, Germany, and Portugal yields annual biomass of 10,000 and 5,000 metric tons, respectively (Qin et al., 2023). The total amount of microalgal biomass produced worldwide is now close to 20,000 metric tons (Ciani et al., 2021; Qin et al., 2023). Table 4 shows the biomass concentrations yielded from different microalgae species cultivated in various systems. PBRs have better control of cultivation conditions as well as higher biomass productivity of 2–8 mg/L as compared to open systems that can produce 0.1–0.2 mg/L biomass (Ahmad et al., 2021).

3.2 Wastewater microalgae cultivation

Bioremediation, where strains can clean up fouled lands resulting from industrial pollution, has also been a topic of interest (Martínez-Ruiz et al., 2022). Cultivating microalgae in various types of wastewater, such as municipal, industrial, or agricultural, can remove wastewater residues such as nitrogen, phosphorus, and carbon (Costa et al., 2019); these compounds support the growth of microalgae (Leong et al., 2021; Wang and Zhang, 2022; Sundaram et al., 2023). Different microalgae species are used for treating specific types of wastewater. For agricultural wastewater, species such as *Leptolyngbya* sp. are commonly used, while in the case of municipal wastewater, *Euglena viridis* is an effective (Satya et al., 2023). Some microalgal species are versatile in bioremediation industrial wastewater that contains high organic loads, as they are capable of performing chemohetetrotrophic or mixotrophic pathways (Leong et al., 2021). Such species include *Geitlerinema* sp., *Galdieria sulphuraria*, *Eucheuma denticulatum*, and *Chlorella vulgaris* (Satya et al., 2023).

The microalgal-bacterial system is a promising wastewater bioremediation alternative (Leong et al., 2020). Microalgae and bacteria can depend on each other symbiotically, where microalgae take up carbon dioxide and convert inorganic into organic while releasing oxygen, which bacteria consume along with the organics (Zhao et al., 2023). In this manner, a newly designed sequential flow baffled microalgal-bacterial photobioreactor was integrated to study the impact of growing the microalgal species, *Chlorella vulgaris,* with a bacterial culture, resulting in a maximum biomass production of 792 mg/L and nitrogen removal efficiency enhanced from 40.91 to 96.38% (Leong et al., 2021, 2022). Therefore, cultivating microalgae on wastewater offers a viable option for lowering the cost of microalgae while simultaneously producing algal biomass as a feedstock for the production of biofuels, fertilizers, animal and fish feed, and other bio-based compounds (Wang and Zhang, 2022; Wang et al., 2022).

3.3 Artificial intelligence in algal resource optimization

Artificial Intelligence (AI) can substantially enhance the productivity of microalgal cultures. Through AI, the optimization of cultivation conditions becomes more precise and comprehensive, enabling higher accuracy in the identification, classification, and quantification of algal strains and their growth dynamics (Peter et al., 2023). This advancement paves the way for the development of automated cultivation systems. Such systems can potentially reduce the costs associated with the harvesting and extraction of bioproducts, increasing the efficiency and economic viability of microalgal biotechnology applications (Long et al., 2022). The use of AI in the microalgae industry is rising with the development of AI technology and the need for sustainable resources. Figure 2 shows the current challenges and AI-driven solutions in the algal industry. AI often involves the use of large datasets, digitalization, and automation for increased efficiency in producing bioactive ingredients (Chong et al., 2023). Algal oil is considered one of the main solutions for renewable energy, but its application faces limitations related to sunlight penetration, poor cultivation dynamics, relatively low yield, and the high cost of industrial harvesting (Chen et al., 2012; Milledge and Heaven, 2013; Slocombe et al., 2021).

Recently, a model was developed to overcome these limitations, where different microalgae were cultured, and their growth was monitored with a mobile spectral imager. By introducing absorbance spectra of the cultured microalgae and simulating pairwise mixtures of them, they trained and validated a one-dimensional convolution neural network. This approach minimized extensive sample processing and allowed for automation, facilitating the microalgal culture analysis

TABLE 2 Comparison of open and closed cultivation systems.

Cultivation systems	Characteristics	Used in/for	Factors to be considered	Advantages	Disadvantages
Open	• Shallow ponds (10–50 cm depth). • Built-in concrete or simply carved from the ground. • It can be recovered by a plastic liner made of high-density polyethylene (HDPE) or polyvinylchloride (PVC). • Can be carried in covered (greenhouse) or open places. • Can use artificial basins and natural water bodies.	• Microalgae products industry: ○ Bioethanol. ○ Biodiesel. ○ Biooil. ○ Biogas. ○ Biohydrogen. ○ Protein ○ Beta carotene ○ Astaxanthin. • Wastewater treatment.	• Pond depth • Mixing • Gas transfer and CO_2 delivery • Light availability • Temperature	• Cheap • Simple • Low energy. • Easy to scale up. • Does not compete with agricultural land.	• Low cell concentration. • Rainwater runoff impact. • High risk of contamination. • Difficulty in controlling growth parameters. • High water loss due to evaporation. • Low CO_2 supply. • Large land area required. • High sensitivity to local weather conditions. • Difficulty in harvesting. • Poor light utilization and distribution. • Difficult to grow for long periods.
Closed	• Closed vessel that helps microalgal cells to carry out photosynthesis in artificial illumination or sunlight as the source of energy • Materials can be glass or polymer.	• Cultivate monoculture of microalgae in axenic enclosed PBRs in the production of biochemical and high value metabolites.	• Light distribution, intensity and quality. • Temperature. • Nutrients. • Carbon Dioxide (CO_2) • pH and salinity. • Mixing • select appropriate materials and conditions • Maintain the humidity.	• Compact size provides more efficient land usage. • Controlled culture conditions and growth parameters. • Little or no dependency on the climate. • Low contamination risk. • Low water evaporation rate. • High microalgal density, concentration, and biomass productivity.	• Limited scalability. • High construction, capital, and operating costs. • Shear stresses may damage the cells. • Chances of overheating and biofouling. • Accumulation of oxygen can decrease the biomass yield. • The formation of algal biofilms causes fouling of PBR surfaces hindering the light penetration.

Characteristics, factors, advantages, and disadvantages of open and closed cultivation systems are provided in the table.
Source: Morales et al. (2019), Chowdury et al. (2020), Tan et al. (2020), Ahmad et al. (2021), Moore (2021), and Wang and Zhang (2022).

(Otálora et al., 2023). Mutual shading is one of the main factors limiting large-scale algal cultivation due to exponential light attenuation along the optical depth gradient (Nwoba et al., 2019). Sustaining optimal growth and minimizing mutual shading can be achieved by using machine learning (ML) to inform the design of semi-continuous algal cultivation (SAC). Aggregation-based sedimentation (ABS) strategies can achieve low-cost biomass harvesting and economical SAC. Based on model predictions, the National Renewable Energy Laboratory (NREL) estimated that a minimum biomass selling price (MBSP) of 281$ compared to 1,227$ per ton for open pond algal cultivation in 2019 (Long et al., 2022). These AI-mediated advances may eventually prove to be the deciding factor in bringing microalgae to successful and sustainable commercialization.

Artificial neural networks (ANN) and support vector machines (SVMs) are ML algorithms used for classification and regression tasks. ANNs resemble biological neural networks in terms of performance, created as extensions of mathematical models of human neural biology. Neural networks have the capacity to process large datasets with adaptive learning, allowing pattern association, classification, clustering, and prediction (Thakur and Konde, 2021). ANN was used to predict the growth of polyculture microalgae in semi-continuous open ponds with different input parameters. The best combination of weight and bias was selected during the training process, resulting in effective and precise prediction of microalgae growth with high accuracy ($R^2 = 0.93$). They identified solar irradiance and temperature as key variants to estimate microalgal growth (Noguchi et al., 2019). The powerful and flexible SVM ML model is capable of minimizing complexity and noise and enhancing the generalization performance of a network by defining the hyperplanes that best separate data into different classes (Elkiran et al., 2019). It has been used in hydro-environmental studies, more specifically for wastewater treatment plant performance analysis (Nourani et al., 2018; Elkiran et al., 2019). SVMs were also used for fucoxanthin extraction optimization and antioxidant activity quantification. Fucoxanthin is a carotenoid with various beneficial medicinal properties for human wellbeing (Chong et al., 2023). In addition, SVMs can predict the growth behavior of microalgae in outdoor cultivation. The Fraunhofer Institute of Interfacial Engineering and

TABLE 3 Suspended and attached cultures.

Cultivation systems	Types	Culture type	Advantages	Limitations
Open	Lakes			
	Ponds			
	Circular ponds			
	Inclined surface systems			
	Raceway ponds	Suspended	• Easily to obtain the growth sources (light or nutrients) leading to increasing biomass.	• High water demand. • Low biomass productivity • High energy. • High cost for harvesting.
Closed	Tubular PBR			
	Vertical column bioreactor			
	Flat-plate PBR			
	Flat plate algal biofilm PBR	Attached	• Simple harvesting via scrapping. • High nutrient concentration. • Occupies less space and can be three-dimensional cultivation. • Simple biomass recovery processes • Low cost.	• Biomass accumulation. • Microalgae would generally lose their ability to utilize high-illumination. • Long-time absence of light leads to low oxygen and a decrease in chlorophyll content.
	Capillary-driven photobioreactor			
	Attached cultivation system with multiple vertical surfaces			
	Drum biofilm PBR			
	Multi-plates attached PBR			
	Revolving algal biofilm PBR with a trough reservoir			

Cultivation systems can be classified into two types of culture; suspended and attached. Their advantages and limitations are presented in this table.
Source: Lin-Lan et al. (2018), Tan et al. (2020), and Zhao and Huang (2021).

Biotechnology used the SVM-based model to predict the growth rate of *Phaedactylum tricornutum* with a correlation coefficient of 88%, a significant advancement toward superior predictive models for microalgae production (IGB, 2023).

Biochar is a carbon-rich product of biomass produced through pyrolysis from waste residues and can also be produced from microalgae (for example, *Chlorella vulgaris*), yielding a potential clean technology for carbon sequestration and microalgal biorefinery (Yu et al., 2018). Machine learning (ML) libraries based on the gradient boosting algorithms (eXtreme Gradient Boosting; XGB) were implemented to predict biochar yields from algae. By achieving a high regression coefficient ($R^2 = 0.84$) between experimental and model predictive biochar yields, they showed that it could be effectively used to estimate biochar yield from biomass conversion. They determined the key parameters (H/C, N/C, Ash, pyrolysis temperature, and time) for deciding biochar yield from algal culture (Pathy et al., 2020). The XGB model evaluated 2^{13} input combinations and identified key parameters (such as pyrolysis temperature, carbon, and ash content). The model can optimize production yield quantitatively and qualitatively (Pathy et al., 2020). XGBoost and other gradient-boosting models were used to predict harmful algal blooms (HABs). HABs are a serious threat to aquatic ecosystems and human health; therefore, managing and controlling their spread is crucial (Ahn et al., 2023).

4 Strain optimization

Can a promising isolate be further improved? Random mutagenesis and genetic engineering are examples of useful techniques adopted for microalgal strain improvement (Carino and Vital, 2022). Random mutagenesis is a useful approach for creating more productive strains, with the advantage of not requiring extensive knowledge of microalgal genetics (Trovão et al., 2022). Microalgal enhanced through random mutagenesis could alter biomass generation, production of target compounds, and strain optimization that leads to increased tolerance of a wider range of environmental conditions.

The process of random mutagenesis involves subjecting microalgae cells to chemical or physical mutagens, leading to the generation of a diverse population of mutants with distinct genetic and phenotypic characteristics (Fathy et al., 2023). Examples of chemical mutagens are alkylating agents, Base Analogs (BAs), AntiMetabolites (AMs), and Intercalating Agents (IAs) while the physical mutagens include UV radiation, ionizing radiation, Atmospheric and Room Temperature Plasma (ARTP), and laser irradiation (Bleisch et al., 2022).

4.1 Strain optimization through UV mutagenesis

UV irradiation is a safer, more rapid, and more effective mutagenesis method in comparison to chemical mutagenesis methods (Sivaramakrishnan and Incharoensakdi, 2017; Carino and Vital, 2022). Based on its wavelengths, UV light is classified into low (UV-A, 320–420 nm), middle (UV-B, 280–320 nm), and high (UV-C, 180–280 nm) energy (Cao X. et al., 2021). UV-C is more efficient for microalgae compared to UV-A or B in terms of its mutagenesis effects (Sivaramakrishnan and Incharoensakdi, 2017). UV-A produces reactive oxygen species and causes indirect

TABLE 4 Biomass of cultivated microalgae species.

Cultivation systems	Type	Cultivated species	Concentration (g/L)	References
Open	Open raceway	*Chlorella vulgaris*	0.5, 1, and 1.67	Morales et al., 2019
	Open raceway	*Nannochloropsis* sp.	0.35, and 0.5	
	Open raceway	*Scenedesmus dimorphus*	0.47	
	Open raceway	*Tetraselmis* sp., *Cyclotella* sp., *Dunaliella* sp., and *Phaeodactylum tricornutum*	1.4	
	Open raceway	*Staurosira* sp., and *Desmodesmus* sp.	0.36, 0.44, and 0.62	
	Open raceway	*Scenedesmus dimorphus*	1.5	
	Open raceway	*Dunaliella salina*	0.4	
	Open raceway	*Spirulina, Isochrysis,* and *Chlorella*	10	
	Open raceway	*Scenedesmus obliquus*	0.43	
	Open raceway	*Scenedesmus dimorphus*	0.5	
Closed	PBR	*Phaeodactylum tricornutum*	3.4	Morales et al., 2019
	PBR	*Nannochloropsis salina*	-	
	Flat plate PBR and tubular PBR	*Nannochloropsis* sp.	2.7, and 1.02	
	PBR	*Chlorella vulgaris*	8.3	
	PBR	*Scenedesmus dimorphus*	4	
	Tubular PBR	*Phaeodactylum tricornutum*	0.2–12	Ahmad et al., 2021
	Tubular PBR	*Nannochloropsi s salina*	7.2	
	Column PBR	*Chlorella* and *Stigeoclonium*	0.49–0.84	
	Column PBR	*Dunaliella tertiolecta*	19.78	
	Flat plate PBR	*Botryococcus braunii*	96.4	
	Flat plate PBR	*Chlamydomonas reinhardtii*	4.5	
	Sequential-flow bubble column PBR	*Chlorella vulgaris*	7.09	Dasan et al., 2020

Examples of cultivated microalgae species detailing their biomass concentrations in various cultivation systems are provided in this table.

damage to the cells by damaging DNA, proteins, and lipids, while UV-B and UV-C cause direct damage to the DNA by forming pyrimidine dimers in adjacent base pairs (Sydney et al., 2018). UV-C irradiation is optimal for methods utilizing random mutations followed by selection, such as those used for microalgae (Fathy et al., 2023).

Mutations caused by UV-C radiation are related to the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6–4) pyrimidinone dimers [(6–4) photoproducts] (Bleisch et al., 2022). During this process, it is essential to prevent light-induced (300–600 nm) DNA repair via photoreactivation (Ha and Bhagavan, 2022) by covering the UV-irradiated plates with foil immediately after exposure and incubating them in the dark (Carino and Vital, 2022) for 24–72 h, depending on the cell cycle period. Photoreactivation, which is mediated by photolyase, is thought to be the major DNA repair pathway for CPDs and (6–4) photoproducts in higher plants where photolyases bind specifically to these DNA lesions and remove them directly by absorbing light in the 300–600 nm range (Ha and Bhagavan, 2022).

Exposing microalgae to UV light increases the levels of saturated fatty acids and monosaturated fatty acids and forms antioxidants, which could be the reason for enhanced polyunsaturated fatty acids synthesis (Udayan et al., 2023). Random UV-C mutagenesis performed on *Chlorella vulgaris* resulted in two isolated mutants with a significantly enhanced total lipid content (75%, >1.3-fold increase; Carino and Vital, 2022). Studies employed UV radiation in *Chlorella vulgaris* and *Scenedesmus obliquus* achieved substantial 2.4- and 2.39-fold increases, respectively, in lipid content when compared to the control strains (Fathy et al., 2023). Table 5 shows other examples of applying UV radiation to enhance biomass and lipid productivity in different microalgae strains. Significantly, microalgal strains improved using the above random mutation methods are not labeled as genetically modified organisms (GMOs) since no foreign genetic material is introduced into their genomes (Trovão et al., 2022).

However, while non-targeted mutagenesis introduces random changes in the genome, the results are usually unpredictable, often leading to mutants with lower yields than the parental strain (Yi et al., 2018). Rare random mutations with positive effects can be isolated if a successful screening method is applied (Yi et al., 2018). Figure 3 illustrates combining classical

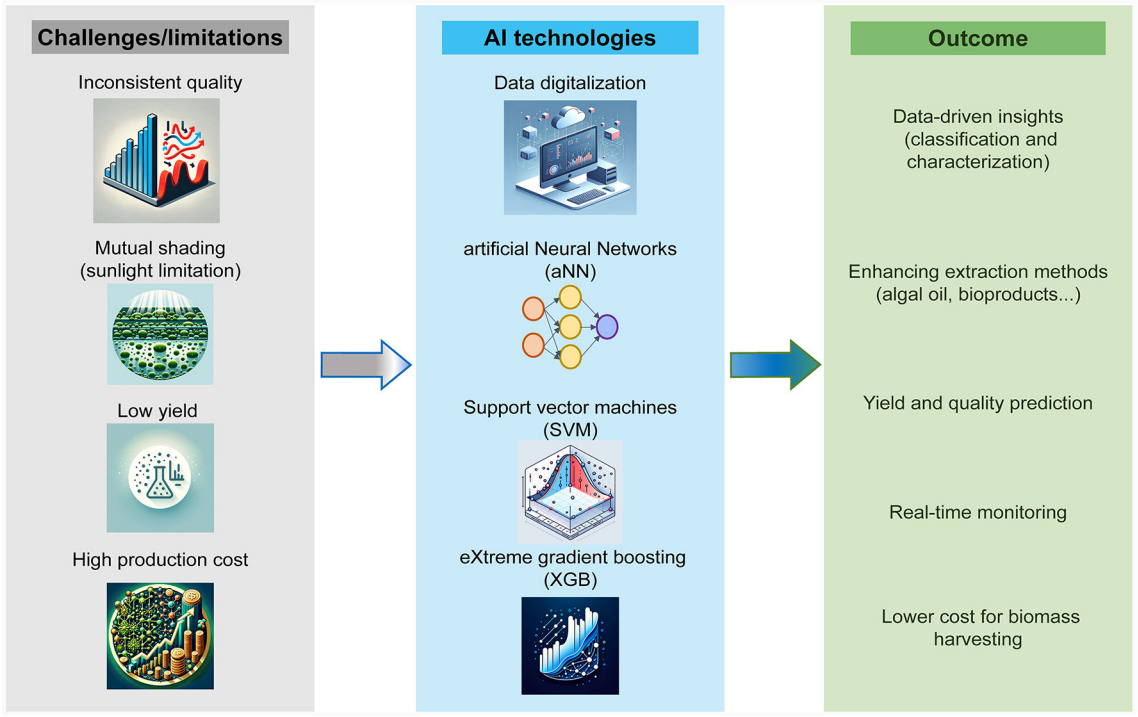

FIGURE 2
Challenges and AI-driven solutions in the algal industry. The diagram illustrates the challenges faced in producing high-value bioactive products from algae (highlighted in gray). It also showcases the AI techniques employed to address these challenges (highlighted in blue) and the anticipated results of these interventions (highlighted in green).

random mutagenesis with high-throughput screening via FACS which has become the gold standard for generating strains with a high growth rate and productivity, stress tolerance, and resistance to common pests (Arora and Philippidis, 2021). FACS quantitatively measures the optical characteristics of each single cell passing through a focused light beam when coupled with fluorescent labeling; the technique allows for differentiating cell subpopulations in various physiological states (Sibanda and Buys, 2017).

Applying random mutagenesis prior to FACS has been reported to isolate lipid-rich mutants of several different microalgal species (Pereira et al., 2018). For instance, Yi et al. (2018) generated several mutants of the model diatom, *Phaeodactylum tricornutum,* with increased carotenoid accumulation using the chemical mutagens ethyl methanesulfonate (EMS) and *N-methyl-N'-nitro-N*-nitrosoguanidine (NTG) alkylating agents. They then screened ~1,000 strains using a fluorescence-based high-throughput method, and five selected mutants had 33% or higher total carotenoids than the wild-type (Yi et al., 2018). Similarly, Sharma et al. (2015) and Abdrabu et al. (2016) applied repetitive rounds of UV radiation on *Chlamydomonas reinhardtii* (CC-503) and selected high lipid accumulated mutants by FACS (Sharma et al., 2015; Abdrabu et al., 2016).

Microalgal compounds can be analyzed by ultra-high-pressure liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS; Lee et al., 2018). It is a rapid and sensitive analytical technique for detecting and identifying metabolites in biological samples (Cao X. C. et al., 2021). This technique has been successfully used to determine and identify intact polar and neutral lipid molecular species among lipidomes (Castro-Gómez et al., 2017). Confocal Raman microscopy (CRM) is a non-invasive method for locating and examining lipids, pigments, and carbohydrates in the algal research (Scalfi-Happ et al., 2011; Sharma et al., 2015). It allows real-time monitoring and examination of living cells without labeling; thus, Raman gives extensive chemical information without damaging or labeling samples (Zhang et al., 2021).

5 Future perspectives and challenges

Microalgae have emerged as a promising group of organisms for pursuing sustainable solutions, specifically in bioproducts, biofuels, and consumer nutritional supplements. The recent data availability and advances in high-throughput techniques helped better understand and characterize the biology of microalgae (Kumar et al., 2020; Villanova and Spetea, 2021; Helmy et al., 2023).

Using systems biology approaches and artificial intelligence can revolutionize the industrial application of microalgae (e.g., algal oil and high-value bioactive products) by optimizing their growth and enhancing product quality. The application of artificial intelligence methods in microalgae research has enabled the definition of the influence of input parameters on cultivation

TABLE 5 UV mutagenized microalgal species.

UV mutagenesis			Selection	Algal strain	Biomass	Lipid		
Type	Distance (cm)	Period (min)			Production (mg/L/day)	Production (mg/L/day)	Increase compared to WT (%)	References
UV-C, 30 W	25	45, 60, and 75	Survival	*Chlorella* sp. 042	50.7, 61.8, and 51.1	27.2, 34, and 20.7	60.5, 55, and 40.6	Rachmayati et al., 2020
UV-B, 1.5 W/cm^2	3	5	Survival	*Chlamydomonas reinhardtii*	ND	79.9* mg/L	11.3	Sydney et al., 2018
		30		*Micractinium inermum*	ND	226* mg/L	ND	
UV-C, 30 W	15	24	Survival	*Chlorella* sp.	30.1	32.4*	28.1	Liu et al., 2015
UV-C	40	0.5	Iodine staining method	*Chlorella vulgaris*	25.3 and 27.3	ND	17 and 50	Carino and Vital, 2022
UV-C, 15 W	50	20	Survival	*Scenedesmus obliquus*	53.32	8.76	24.8	Qi et al., 2018
UV-C, 3.4 W/m^2	15	40	Biomass and lipid content determination	*Chlorella* sp.	1970*	9.5* mg/L	48	Sivaramakrishnan and Incharoensakdi, 2023
30 W	25	30	Nile Red fluorescence	*Chlorella* sp. 042	30.8 and 25.62	11.27 and 11.20	35.15, and 43.85	Rahman et al., 2020
UV-C, 15 W	34	0.5	Nile Red fluorescence	*Chlorells sorokiniana* SAG 211–32	266 and 293	3.5 and 3.3[&]	40 and 30	Vigeolas et al., 2012
		2		*Scenedesmus obliquus* SAG 276–10	318 and 272	13.7 and 19.6[&]	Unchanged	

The table below lists examples of different microalgae species that were subjected to UV radiation to enhance their biomass and lipid productivity.
ND, No Data.
*mg/L.
[&]pg lipid per cell.

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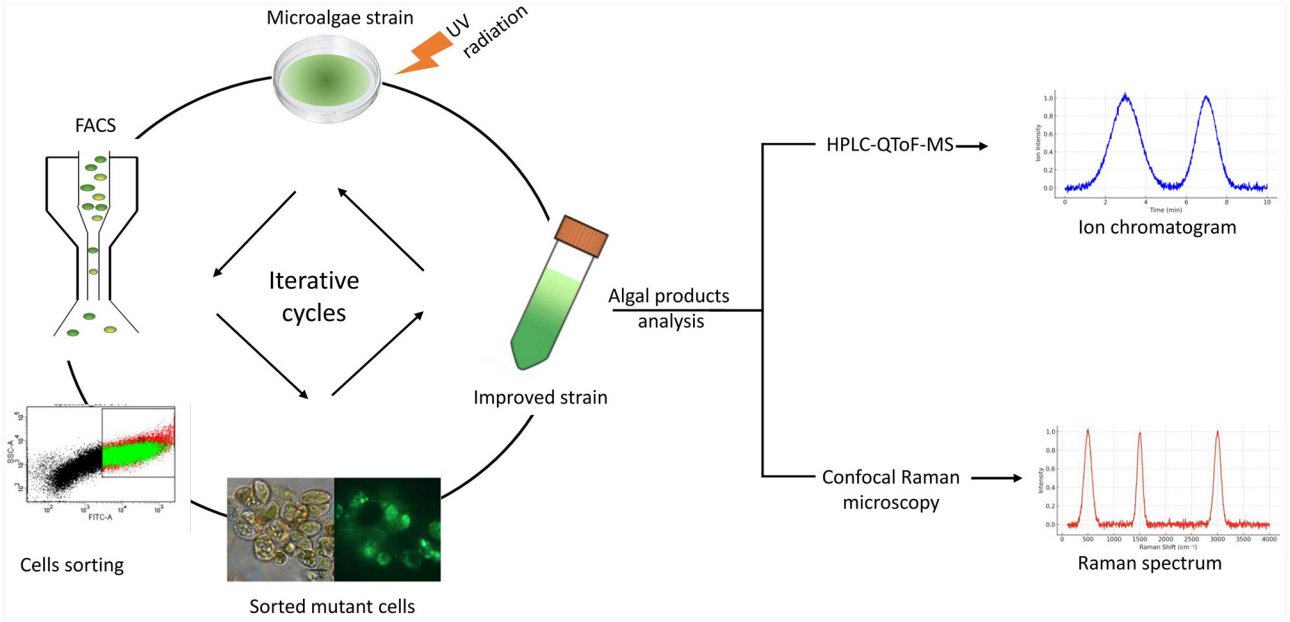

FIGURE 3
Iterative cycles of UV mutagenesis combined with fluorescence-activated cell sorting (FACS). A diagram of the workflow for applying UV radiation to microalgae strains combined with live-cell sorting by FACS to improve strain productivity is shown. These steps can be repeated to obtain mutants with the desired phenotypes. Analysis of the cellular metabolites of the sorted algal populations can be done by ultra-high-pressure liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) or by confocal Raman microscopy.

conditions and variables, resulting in high prediction accuracy that significantly enhances the efficiency and optimization of algal cultivation systems and industry. While ANNs comprise the go-to algorithms for ML, they are often complemented by other approaches like SVMs and XGBs (Coşgun et al., 2023). At present, ML is an emerging field in algal cultivation. The mentioned methods allow the deciphering of complex patterns and interactions that can help to overcome limiting challenges and achieve sustainability.

With increased environmental and supply chain challenges, microalgae are expected to gain interest globally. In addition to their prospective applications, microalgae are great candidates for addressing pressing issues like wastewater treatment and carbon sequestration. The full potential of microalgae will likely be realized through multidisciplinary research to solve the most critical problems. Of these, advances in cultivation systems and strain engineering will be vital. Importantly, integrating multi-omics datasets through AI approaches can provide critical solutions to enhance productivity.

6 Conclusion

This review examined the aspects of microalgal isolation, cultivation, and strain optimization, focusing on mutagenesis screens and the use of artificial intelligence in optimizing cultivation. Biotechnological innovations, such as genome editing tools, will play a vital role when molecular-genetic tools are available for the species. Furthermore, comprehensive techno-economic analyses considering scalability and sustainability factors will be crucial in guiding the commercialization of microalgae. With concerted efforts, microalgae can significantly contribute to global sustainability initiatives for food, fuel, and nutraceuticals.

Author contributions

KS-A: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. AA: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. SD: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. DN: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. DA-K: Conceptualization, Formal analysis, Visualization, Writing – review & editing. J-CT: Conceptualization, Writing – review & editing.

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Icon images in Figures 2, 3 were obtained from: https://chat.openai.com (GPT-4) using the DALL-E 3 plugin. Claude-2 (https://www.anthropic.com/index/claude-2) was used to improve the flow and readability of the text, Claude-2, Quilbot (https://quillbot.com/), and Grammarly (https://www.grammarly.com/) were used as proofreading tools.

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