Investigating the Impact of Amines on Freshwater Microalgae Relevant for Sustainable Carbon Capture Systems

Dipesh [1], N. C. Gupta [1,*], Ruchika Tanwar [1], Anubha Kaushik [1], and Ekansh Saigal [2]

[1] University School of Environment Management, GGS Indraprastha University, Dwarka, New Delhi-110078, India
[2] University School of Biotechnology, GGS Indraprastha University, Dwarka, New Delhi-110078, India
Email: dipeshdubdey132@gmail.com (D.); ncgupta@ipu.ac.in (N.C.G.); r.tanwarr@gmail.com (R.T.); aks.es.10@gmail.com (A.K.); saigalekansh06@gmail.com (E.S.)
*Corresponding author

Manuscript received September 19, 2025; revised November 9, 2025; accepted December 22, 2025; published August 17, 2026

***Abstract*—The Post-Combustion Carbon Capture (PCCC) process relies on aqueous amine solutions to chemically absorb CO_2 from flue gas, and it has gained rapid acceptance as an effective method for mitigating carbon emissions. However, during this process, the amines and their derivatives escape into the environment. Since the degraded solvents are considered ecotoxic and carcinogenic, they may potentially harm aquatic systems and have broader ecological and health repercussions for humans. To assess the toxicity of various amines and their derivatives, including formamide, acetamide, and Monoethanolamine (MEA), we have conducted ecotoxicity tests on the freshwater unicellular algae *Chlorella pyrenoidosa* under controlled conditions. The research highlights the significance of both acute and chronic exposure to key biomarkers, revealing essential insights into potential sublethal effects and tolerance levels of each amine solvent. These factors are crucial to algal efficiency and growth, underscoring the importance of sustainable solvent use in carbon capture, utilisation, and storage technologies.**

***Keywords*—amine-based solvents; toxicity; Carbon Capture, Utilization and Storage (CCUS); *Chlorella pyrenoidosa*; total chlorophyll; algal growth**

I. Introduction

Economies' reliance on traditional fossil fuels for energy production is the primary driver of the spike in worldwide carbon dioxide emissions [1]. Along with rising temperatures and changing weather patterns, the effects of growing atmospheric CO_2 concentrations have already been witnessed in the real world [2]. Reaching net zero and reducing greenhouse gas emissions by 2070 is essential to limiting global warming to 2 °C over pre-industrial levels; yet, reducing global CO_2 emissions is the only way to alleviate these concerns [3, 4]. To meet mid- to long-term CO_2 reduction goals, one technological approach is Carbon Capture, Utilization and Storage (CCUS) [5, 6]. These technologies are intended to stop CO_2 from being released into the environment by directly capturing it from emission sources and utilizing it as raw material for other processes [7, 8]. Amine-based CO_2 absorption is one of the most effective and promising CCUS techniques available [9]. However, degradation of the solvents (amines) used in the absorption process remains an important concern, as it leads to the formation of hazardous by-products, including ammonia, aldehydes, amides, alkyl amines, and nitrosamines [4, 10–12]. These by-products may significantly impact the air and surface water quality in the area. Amine-rich wastewater is categorized as hazardous waste due to its harmful effects on human health and its toxicity to plants, microbes, and marine life [13, 14]. Few studies have corroborated the significant gaps in our understanding of the environmental impacts of amine-rich wastewater generated by carbon capture technologies. Specifically, the toxicity data available on amines and their byproduct emissions into air and water vary widely [15, 16]. Given the paucity of pertinent data, an experimental strategy is needed to assess the effects of amine-based solvents used in the carbon capture process on the aquatic environment and to identify potentially hazardous concentrations. Thus, further studies on the toxicity and occurrence of amines and their byproducts in the freshwater system are imperative.

The present study advances understanding of possible ecotoxicological effects of amines and their byproducts through a detailed, species-specific investigation of three major amines used in solvent mixtures for CCUS technology and quantification of their impacts using three physiological and biochemical parameters of the test microalgae species. Unlike previous studies that generally relied on toxicity endpoints, the novelty of this study lies in examining the concentration- and exposure-dependent growth responses of the important freshwater microalgae species *Chlorella pyrenoidosa* to amines under controlled conditions, providing new and significant insights into the potential ecological hazards of amines and their degradation products to aquatic ecosystems. This can help provide vital databases for developing regulatory standards for amine/degradation product emissions near industrial plants using CCUS technology. Thus, the primary focus of the present work is to assess the impact of different exposure times and concentrations on the microalga using important biomarkers, including cell growth, chlorophyll (a major photosynthetic pigment), and total protein as an index of nitrogen metabolism. This particular microalga was chosen for the study due to its ecological significance and dominance as a major primary producer in the food webs of aquatic ecosystems [17, 18]. Understanding of how these amines affect microalgae may help in developing mitigation strategies.

II. Literature Review

Global CO_2 emissions have increased significantly in recent years, largely due to the combustion of fossil fuels. For example, rising fossil fuel use has been the main driver of India's continued rise in CO_2 emissions. CCUS provides a workable way to reduce the carbon footprint of industrial operations by absorbing and storing CO_2 or using it to create

green chemicals [5]. CCUS has been widely acknowledged and supported by major international organisations, including the Paris Agreement, the Kyoto Protocol, the Intergovernmental Panel on Climate Change (IPCC), and the United Nations Framework Convention on Climate Change (UNFCCC) [19, 20].

Monoethanolamine (MEA), Methyldiethanolamine (MDEA), Diethanolamine (DEA), Diglycolamine (DGA), Aminomethylpropanolamine (AMP), and piperazine are the major amines explored as solvents in CCUS [21, 22]. The cleaned gas, which is subsequently released from thermal power plant stacks, dissolves in precipitation, deposits, and eventually finds its way into natural water bodies such as lakes and rivers. Since the amines utilised in the CO_2 removal process are extremely soluble in water, it is reasonable to assume that solvent-degraded products also exhibit these characteristics [23]. Humans, plants, algae, and microbes in the vicinity of such emission sources may be at risk from amines released into the environment [24]. Amines and their by-products released into the environment may be dangerous and affect ecosystems by altering how living things develop and metabolise these compounds [25]. Nevertheless, there is presently less environmental toxicity data available for amine-based solvents, despite the possibility that their levels in the environment could rise and affect the ecosystem [26]. Generally, these studies rely on theoretical calculations; however, experimental data on the toxicity of by-products to algae remain scarce [27].

Freshwater green algae, as primary producers, play a vital role in aquatic ecosystems. An ecotoxicity evaluation of aquatic life is necessary to assess the environmental threat posed by the newly developed CCUS technology. A study on the ecotoxicity of primary amines and their metabolites has been conducted, emphasising the high toxicity of nitrosamines (Lowest Observed Effect Concentration (LOEC) 0.025 ppm) and nitramines. When evaluated with volatile nitrosamines, fish and invertebrates were less harmed, while algae were quite susceptible [28, 29].

Another work reported the presence of N-nitrosamines in the surface waters of the Pearl River Delta (PRD) in Southeast China, along with the impact of growth and biochemical markers on *Selenastrum capricornutum* in relation to N-nitrosodiethylamine (NDEA), N-nitroso-di-n-propylamine (NDPA), N-nitrosopyrrolidine (NPYR), and N-nitrosodibutylamine (NDBA) and their quaternary mixture [27]. As individual N-nitrosamine concentrations rose from 0.1 mg/L to 350 mg/L, growth inhibition rates rose from around 10% to 60%. The quaternary combination reduced algal growth by 15.6% at ecologically relevant concentrations (1000 ng/L), indicating that the mixture's toxicity exceeds that of its constituent chemicals. Collectively, these studies demonstrate the severe risks of amines and their by-products to aquatic organisms. Each study emphasises the urgent need for robust toxicity testing and regulation to safeguard ecosystems. The most noticeable adverse effect that a particular group of compounds causes in any part of the target environment (drinking water, vegetation, terrestrial fauna, ecosystem types), as well as in the receptor organisms (algae, invertebrates, fish, and humans), should be taken into consideration when establishing safe concentration levels.

Thus, the purpose of this work was to evaluate ecological concerns related to residual by-products in aquatic habitats and to examine the growth inhibition and toxicity of amine by-products on *Chlorella pyrenoidosa.*

III. Materials and Methods

A. Chemicals

The amine-based chemicals, viz., formamide (Central Drug House, CDH), acetamide (CDH), and Monoethanolamine (MEA) (Sigma-Aldrich > 99%), were selected for toxicity assessment. Stock solutions of each amine were prepared with sterile distilled water. Working concentrations were prepared by serial dilution and used to assess their effects on the microalgae.

B. Test Organisms

Chlorella pyrenoidosa, a freshwater green alga, has been selected as the test organism for this research. The green alga was selected for its well-known sensitivity to toxins, which makes it a perfect indicator for toxicity analysis [17]. A pure culture of the test microalgae was obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India.

C. Microalgal Nutrient Media

The microalgae culture was cultivated in Tris-Acetate-Phosphate (TAP) media [30], following the specified nutrient composition (per litre):

- TAP stock nutrient: NH_4Cl (16 g), $MgSO_4 \cdot 7H_2O$ (4 g), $CaCl_2 \cdot 2H_2O$ (2.65 g).
- Phosphate Buffer: 28.8 g of K_2HPO_4 and 14.4 g of KH_2PO_4.
- Hunter's trace element: $FeSO_4 \cdot 7H_2O$ (4.99 g), $ZnSO_4 \cdot 7H_2O$ (22 g), H_3BO_3 (11.4 g), $MnCl_2 \cdot 4H_2O$ (5.06g), $CuSO_4 \cdot 5H_2O$ (1.57g), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1.10 g), $CoCl_2 \cdot 6H_2O$ (1.61 g), $Na_2EDTA \cdot 2H_2O$ (50 g).
- Tris base (2.42 g).
- Glacial acetic acid (1.0 mL).

D. Preparation of Seed Culture

Microalgae culture was prepared under aseptic conditions. The TAP medium was meticulously prepared using Milli-Q water, and the pH was precisely adjusted to 7.0 ± 0.2 using 1N HCL or NaOH. The media were autoclaved at 121 °C for 15 min to ensure optimal conditions for the intended application. After reaching room temperature, inoculation was performed in a laminar airflow hood to prevent contamination. The culture was maintained at a light intensity of 3000 lux, using LED lights programmed to provide 12 h of light followed by 12 h of darkness at 27 ± 3 °C in a custom-designed incubator. *Chlorella Pyrenoidosa* grows well at 25–35°C and under a 12:12 light:dark photoperiod, supporting robust growth and cellular recovery and reducing metabolic stress [31–33].

Photoperiods of 16:08 light:dark favour higher biomass production [34, 35]. The cultures were shaken on a rotatory shaker twice a day for 30 min at 130 rpm to avoid sticking. Throughout the exposure period, cultures were actively mixed to prevent cell aggregation. The microalgae were grown for 7 days to prepare them as a seed culture for experimentation. Strict aseptic techniques and consistent mixing were employed, which are essential for minimising

experimental variability. The overall experimental design and workflow are illustrated in Fig. 1.

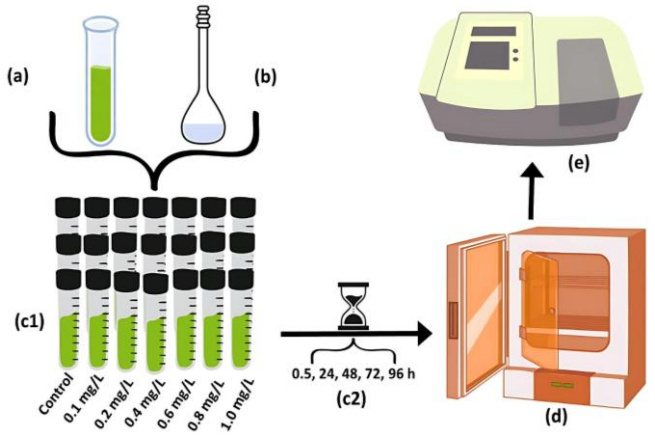


Fig. 1. Schematic of ecotoxicity study of amine-based solvents on algae (a) Algal suspension culture (b) Stock solution of amines (c1) Exposure of algae to different concentrations of amines (c2) Varying exposure time of amines to algae (d) Algal growth chamber with controlled light and temperature (e) UV-vis spectrophotometer for bioparameter analysis.

E. Exposure to Varying Amine Concentrations

The concentrations of the amines varied from 0.1 mg/L to 1.0 mg/L. The range of chemicals was carefully chosen to represent the likely environmental exposure limits and their impacts on water ecosystems [36].

F. Duration of Exposure to Amines

Cultures were exposed to the three amines, MEA, Formamide, and Acetamide, at concentrations of 0 mg/L (control), 0.1 mg/L, 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L, and 1 mg/L, respectively, for varying time periods, viz. 0.5 h (30 min), 24 h, 48 h, 72 h, and 96 h. The exposure range provides a comprehensive understanding of microalgae's response to amines over time.

Each exposure was carried out in sterile culture tubes containing 5 mL of algal culture. The culture tubes were maintained at 27 ± 3 °C, replicating the conditions used for the seed cultures. The pH levels were carefully monitored at both the start and the end of the exposure, with no adjustments made during the test to avoid additional stress. All treatments, differing in amine concentrations and exposure durations, included three replicates. Each replicate was treated independently, guaranteeing repeatability and reliability of the results.

G. Analytical Methods

1) Measurement of algal biomass

To assess biomass, the culture was measured for optical density at 680 nm using a Shimadzu UV-1800 Portable Spectrophotometer. The research studies [37, 38] have verified a strong correlation between biomass and optical density at 680 nm, as shown by a standard curve, thereby confirming that absorbance measurements are a reliable means of studying algal growth. The sample was prepared by centrifuging the algal suspension at 5000 rpm, rinsing with deionized water, and oven-drying the pellets at 80 °C for 24 h to attain a constant weight.

$$y = 0.0258x + 0.0003 \quad (1)$$

The linear regression equation obtained has an R^2 value of 0.994. Using Eq. (1), the algal biomass was calculated.

2) Estimation of total chlorophyll pigment

Total Chlorophyll was estimated using the hot extraction method [39]. The microalgal suspension was centrifuged at 5000 rpm for 15 min to pelletize the algal cells. Then, the pellets were washed several times with distilled water to remove any residual media or impurities. The washed pellets were suspended in 10 mL of methanol (95%) and incubated at 60 °C for 30 min. After this, the samples were again centrifuged at high speed (5000 rpm) for 15 min, and the supernatant contained the extracted chlorophyll pigments. Finally, the absorbance of the supernatant solution was measured at wavelengths of 650 nm and 665 nm using a spectrophotometer. The concentration of chlorophyll pigment was then estimated by using the formula mentioned below:

$$\text{Total chlorophyll (mg/mL)} = 2.55 \times 10^{-2}\, OD_{650} + 0.4 \times 10^{-2}\, OD_{665} \quad (2)$$

Using Eq. (2) above, the total chlorophyll was estimated.

3) Estimation of protein concentration

The protein test, as described by *Lowry et al.* [40], was used to evaluate the protein concentrations in *Chlorella pyrenoidosa. Ce*llular lysates were prepared systematically, as they served as the primary source of proteins for subsequent analysis. The three reagent solutions essential for the protein assay were prepared accurately as follows: Solution A, containing 2% sodium carbonate in 0.1 M sodium hydroxide; Solution B, comprising 1% copper sulphate; and Solution C, consisting of 2% sodium potassium tartrate. Then, the main Copper reagent was prepared by combining exact volumes of Solutions A, B, and C as described in the literature. Finally, the Folin-Ciocalteu reagent (Solution E) was diluted to 1 M acid.

After preparing the required reagents, the cellular lysates were carefully mixed with the Copper reagent and incubated at room temperature to form protein-bound copper complexes. Once the incubation period was complete, Folin-Ciocalteu reagent was promptly added to the mixture, and the resulting solution was incubated for an additional 30 min under ambient conditions. Absorbance of the solution was then taken at 600 nm using a UV-vis spectrophotometer. Protein concentrations were estimated by referencing a standard curve produced alongside using Bovine Serum Albumin (BSA).

$$y = 0.9948x + 0.0433 \quad (3)$$

The linear regression equation was obtained with a correlation coefficient of R^2 = 0.9998. Using Eq. (3), the protein concentration of algae was estimated.

H. Statistical Analysis

Microsoft Excel 2019 was used to process the data, and figures were drawn by Origin 2018. The presented values are the mean of three replicates with standard deviation. Error bars representing the standard deviation from the mean provide an effective graphical representation of data variability, enhancing our understanding of the distribution and data reliability. Statistical analysis was carried out using

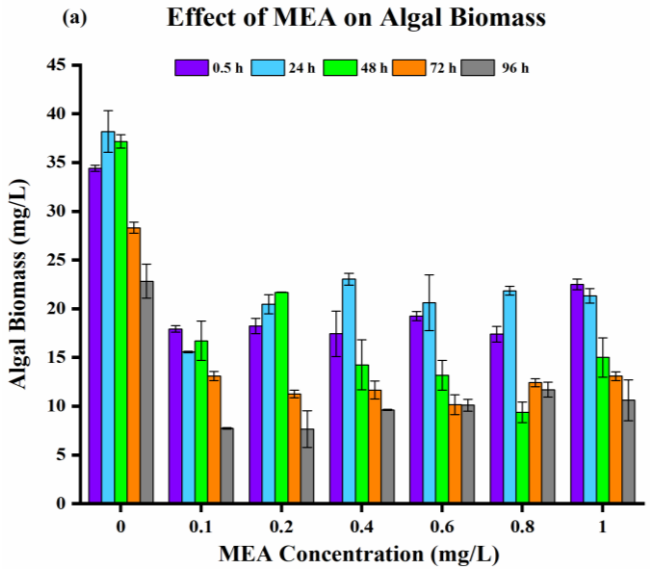
Analysis of variance (ANOVA) in SPSS 27, followed by Tukey's Honestly Significant Difference (HSD) post hoc comparisons to determine significance at $p < 0.05$, which was considered statistically significant and strengthened the reliability of the results.

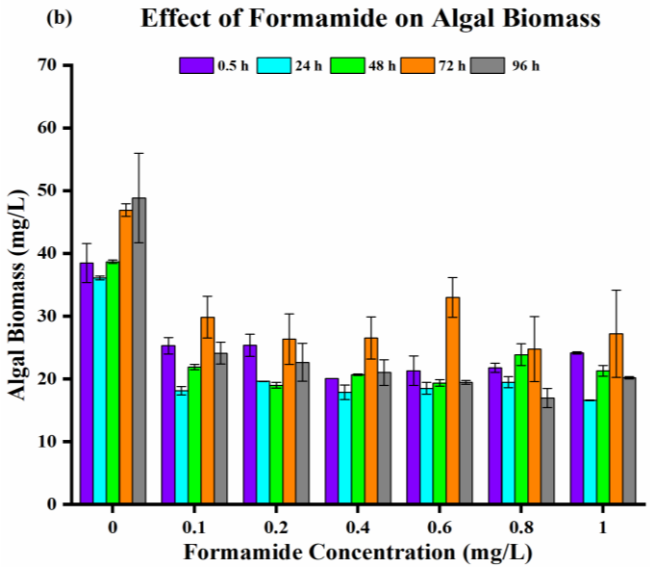
IV. Results and Discussion

To realise the envisioned goals, the results of the research work, including estimates of cell growth dynamics, chlorophyll pigment levels, and protein concentration, have been reported.

A. Effect of Different Amine Concentrations and Exposure Duration on Microalgal Biomass

The algal biomass of *Chlorella pyrenoidosa* exposed to different amines was determined by absorbance using Eq. (1). Since the experiments were performed independently for each amine over time, some variations in culture conditions were likely. Therefore, for each amine, a control set was run in parallel. Fig. 2 illustrates the impact of varying concentrations (0.1–1 mg/L) of (a) MEA, (b) formamide, and (c) acetamide on the cell growth of *Chlorella pyrenoidosa* over a 0.5–96 h period.





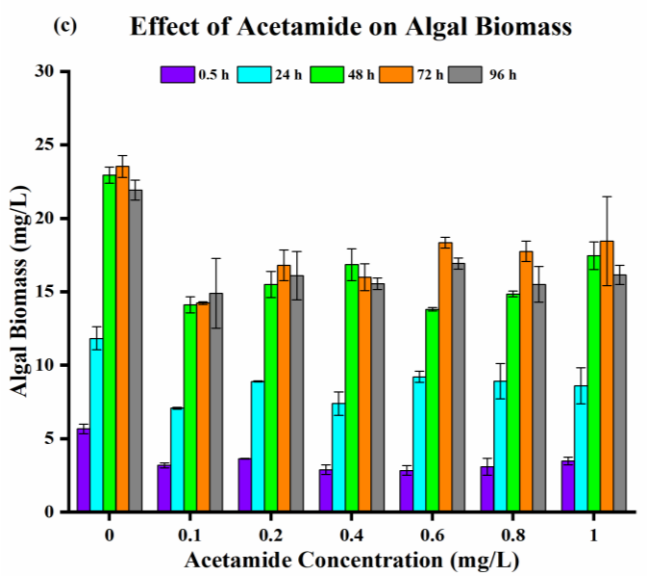


Fig. 2. Biomass variations in *Chlorella pyrenoidosa* exposed to various amine concentrations and different exposure durations: (a) MEA; (b) Formamide; (c) Acetamide.

In the MEA experiment, the control set (0 mg/L) showed rapid growth, attaining 37–39 mg/L biomass in 24–48 h, followed by a later decline. A general decline in biomass was seen in response to increasing MEA dose and exposure time. There was a 28–33% decrease in algal biomass due to increasing MEA dose and exposure. Formamide (Fig. 2(b)) inhibited algal growth by a 40–50% decline in biomass. At extremely low doses, growth was comparable to that of controls, indicating a baseline tolerance. However, when the concentration increased, growth was rapidly suppressed, and biomass declined across all time points.

The growth profile of the microalgae was also affected by increasing the acetamide dose and exposure (Fig. 2(c)), but the decline was relatively less (23%–26%) than in the control. Initial acetamide exposure of 0.5 h resulted in a sharp decline of 50%–60% biomass, but the differences were less with a 25%–45% decline at 24 h, which further narrowed down to 25%–33% decline at 48–96 h, indicating gradual recovery with time.

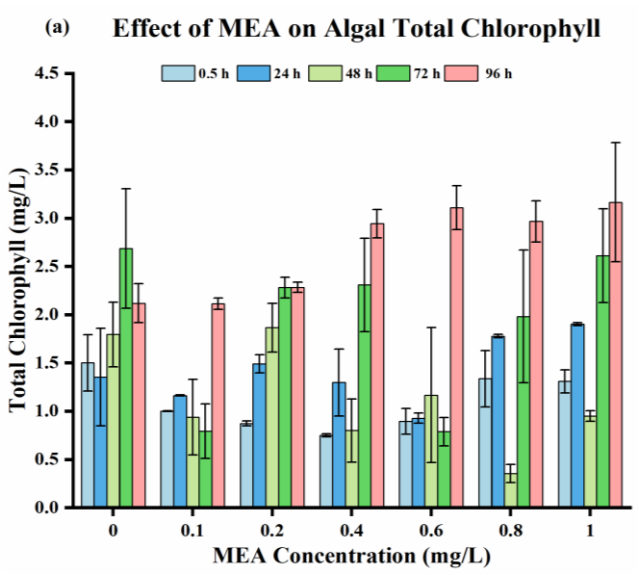
The reduction in biomass of *Chlorella pyrenoidosa* with increasing MEA concentration indicates that amines seem to impose a metabolic burden and cellular stress. MEA can serve as an external carbon and nitrogen source, enhancing algal metabolism at low, controlled concentrations; however, excessive MEA leads to ethanolamine accumulation, membrane degradation, and altered metabolic processes [41, 42]. This supports the 28%–33% decline, suggesting the excess MEA interfered with cellular metabolism, resulting in reduced growth. Formamide is cytotoxic in nature and disrupts hydrogen bonding, making DNA and proteins less stable [43]. It is readily assimilated as a nitrogen source by the majority of the microorganisms [44]. This explains why growth rapidly declines when concentrations reach 0.4 mg/L or higher, followed by a rapid drop, suggesting early stress leading to cell lysis or metabolic collapse. Whereas acetamide showed a sharp decline at 0.5 h, followed by a slow recovery. This matches the microbial response to acetamide, where it initially induces stress and is later metabolized via amidase activity. Amidases convert acetamide to ammonium and acetate, enabling the possible

use of nitrogen source when enzymatic systems are activated [45–46]. This is why the growth inhibition narrowed over time (48–96 h), suggesting acclimation and partial utilisation of acetamide.

The two-way ANOVA results for MEA, Formamide, and Acetamide indicate that algal biomass is significantly affected by exposure duration, concentration, and their interaction. For MEA, time (F = 290.67, p < 0.001), concentration (F = 387.89, p < 0.001), and time × concentration interaction (F = 13.27, p < 0.001) were all highly significant. Tukey HSD confirmed significant differences among all time points, with biomass peaking at 24 h before declining at 48, 72, and reaching its lowest level at 96 h; the control (0 mg/mL) consistently exhibited much higher biomass than all treated concentrations, with several comparisons involving 1.0 mg/mL showing particularly big differences. Formamide exhibited highly significant effects of time (p < 0.001), concentration (p < 0.001), and their interaction (p < 0.001). Tukey's results indicated a continuous increase in biomass from 0.5 h to 96 h, demonstrating that the control biomass was significantly higher than all treatment concentrations, with the lowest at 0.40 mg/mL. Acetamide also showed very significant effects of time (p < 0.001), concentration (p < 0.001), and their interaction (p < 0.001). Tukey's analysis showed that all time intervals differed and that all treated groups had lower biomass than the control. The combined ANOVA and Tukey analyses indicate that biomass is significantly influenced by exposure duration, dose, and their interaction across all three chemicals, with all MEA-, formamide-, and Acetamide-treated groups exhibiting substantially lower algal biomass compared to their respective controls.

B. Effect of Different Amine Concentrations and Exposure Duration on Microalgal Total Chlorophyll

The effects of different concentrations of MEA (0 to 1 mg/L) on the total chlorophyll in *Chlorella pyrenoidosa* during the course of exposure for periods of 0.5 h, 24 h, 48 h, 72 h, and 96 h are displayed in Fig. 3(a). In the control, the chlorophyll content progressively rises up to 72 h from an initial 1.5 mg/L to 2.68 ± 0.62 mg/L, followed by a slight decline after 96 h (2.12 ± 0.20 mg/L), indicating a typical development trend.



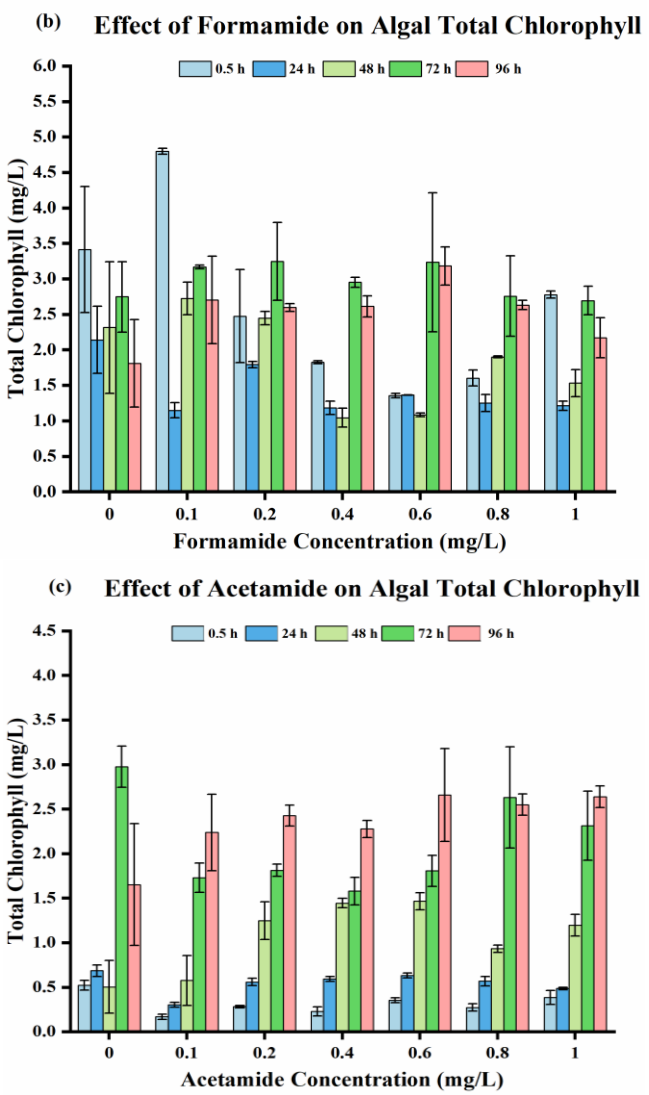


Fig. 3. Total chlorophyll in *Chlorella pyrenoidosa* exposed to various amine concentrations and different exposure durations: (a) MEA; (b) Formamide; (c) Acetamide.

MEA causes suppression of chlorophyll pigments at early exposure times (0.5–24 h), which appears to result from sudden oxidative stress. At moderate concentrations (0.4–0.6 mg/L) during the intermediate phase (48–72 h), pigment formation increases, suggesting that MEA serves as a nutrient source for the algae [47].

Fig. 3(b) illustrates how Formamide affected the concentration of chlorophyll in *Chlorella pyrenoidosa*. Chlorophyll concentration was initially higher at a control concentration of 0 mg/L at all time periods, particularly at 0.5 h and 48 h, indicating stress-free, healthy development. This elucidates how chlorophyll synthesis occurs best when formamide is absent.

The microalgae showed a substantial spike in chlorophyll content to 4.8 ± 0.004 mg/L at a formamide concentration of 0.1 mg/L during the early 30 min examination. However, it decreased to 2.5–3 mg/L as exposure time increased from 24 h to 96 h, indicating an initial stimulatory impact followed by inhibition. At higher doses (0.4–1 mg/L), the microalga showed greater chlorophyll content when exposed to formamide for longer durations than at shorter exposure times, suggesting gradual acclimation to the amine over time, likely as a source of nitrogen, leading to recovery in growth.

Fig. 3(c) demonstrates the effects of acetamide exposure

on *Chlorella Pyrenoidosa* chlorophyll concentration. The chlorophyll content decreased after 30 min of exposure to varying acetamide concentrations when acetamide levels were allowed to increase. This immediate reaction suggests potential toxicity even at low acetamide doses. However, there was a rebound at 72–96 h, with an increase in chlorophyll. Early time points and low-to-mid concentrations showed the largest reductions in algal chlorophyll levels, suggesting a dose-dependent toxic effect. As exposure to acetamide increases, chlorophyll concentration decreases overall, while algal concentration gradually recovers.

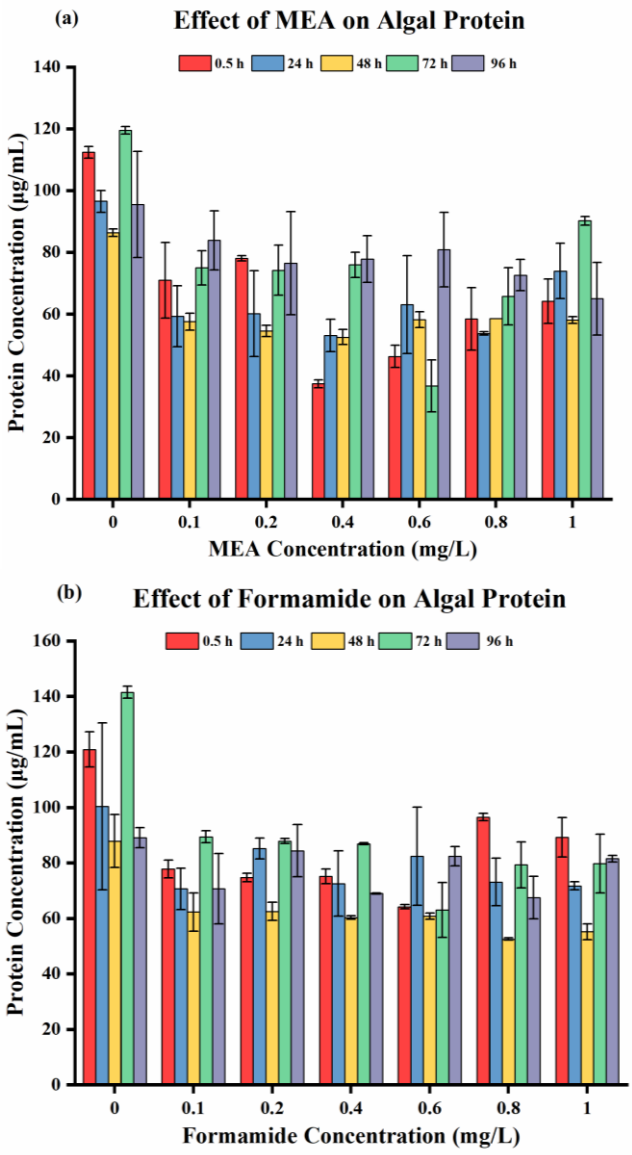
MEA during initial exposure inhibited chlorophyll levels, indicating an immediate amine-induced stress response, consistent with observations that amines can disrupt algal physiology at low to moderate concentrations [47]. At moderate levels (48–72 h), chlorophyll content increases, suggesting a hormetic effect, implying that MEA may function as a moderate stressor, inducing compensatory growth and pigment synthesis [48, 49]. The late phase suggested some metabolic adaptation via the activation of antioxidant defense and chlorophyll biosynthesis pathways [50, 51]. Formamide was first followed by stimulation, then by inhibition, a characteristic pattern of initial activation followed by toxicity. Its ability to disrupt cellular machinery and destabilize metabolic processes, which possibly will explain the sustained reduction in chlorophyll at higher concentrations. The partial recovery at 72–96 h likely indicates acclimation, during which microalgae gradually restore pigment synthesis [52, 53]. Acetamide caused a rapid decline in chlorophyll, suggesting dose-dependent toxicity, as other microalgae respond to chemical or environmental stressors [54]. The recovery of chlorophyll at longer exposure durations suggests that *Chlorella pyrenoidosa* might have activated biochemical pathways that mitigate stress and restore photosynthetic pigments, consistent with established microalgal stress-response mechanisms [55–57].

The two-way ANOVA for chlorophyll levels under MEA, acetamide, and formamide treatments showed strong effects of exposure duration, concentration, and their interaction. In MEA, time (F = 235.25, p < 0.001), concentration (F = 103.82, p < 0.001), and time × concentration interaction (F = 108.87, p < 0.001) all had highly significant influences on chlorophyll levels, with Tukey results showing a continuous rise over time and markedly higher levels at 72 h and 96 h than earlier time points; concentration-wise, 0.40 mg/mL produced the lowest chlorophyll and 1.00 mg/mL the highest. ACT treatment similarly showed significant effects of time (p < 0.001), concentration (p < 0.001), and their interaction (p < 0.001), with Tukey's test showing that chlorophyll levels at 72 h and 96 h were significantly higher than earlier exposures, and that 0.40 mg/mL produced the lowest values. In contrast, several treatment doses differed significantly from the control, indicating a clear dose-time-dependent pattern. Formamide treatment also exhibited significant effects of time (p < 0.001), concentration (p < 0.001), and their interaction (p < 0.001), with Tukey confirming that chlorophyll increased consistently from 0.5 h to 96 h with major differences across intermediate time points; concentration-wise, 0.40 mg/mL again showed the lowest values, whereas the control (0 mg/mL) and 1.00 mg/mL treatments were significantly higher. Overall, the combined ANOVA and Tukey findings across all three chemicals reveal strong, consistent dose-time-dependent increases in chlorophyll, driven by significant main effects and interactions.

C. *Effect of Different Amine Concentrations and Exposure Duration on Microalgal Protein Concentration*

Variations in total protein content of *Chlorella pyrenoidosa* exposed to different MEA concentrations (0–1 mg/L) during the course of 0.5–96 h are displayed in Fig. 4(a). It is evident that the protein content of the microalgae typically declines when given treatment of different MEA concentrations in comparison to the respective control.

During 0.5–48 h, protein content in 0 mg/L MEA varied from 84–112 µg/mL, which fell sharply to 40–75 µg/mL at MEA concentrations of 0.1–0.4 mg/L. Interestingly, there was some recovery in microalgal protein at higher concentrations. It was observed that during the initial period (0.5 h to 48 h of exposure), there is a general decline in microalgal protein concentration across all concentrations. However, exposure for a longer duration of 72–96 h tends to recover protein content in the algal cells. Protein levels declined more sharply on exposure for 48–72 h, indicating cumulative toxicity.



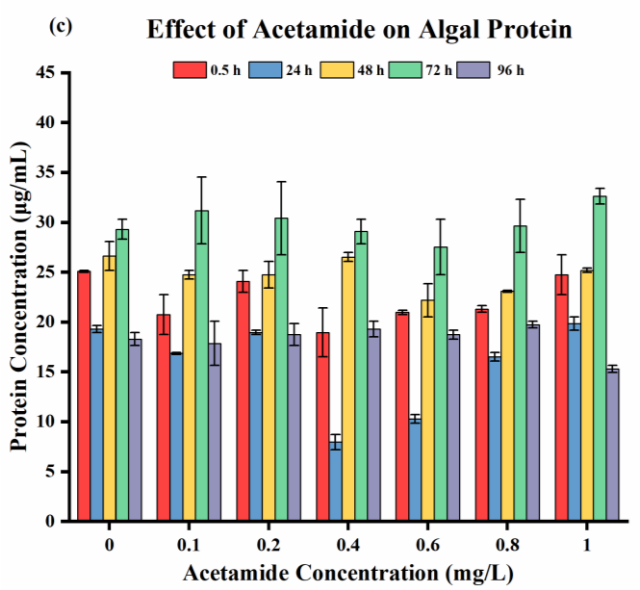


Fig. 4. Protein concentration variations in *Chlorella pyrenoidosa* exposed to various amine concentrations and different exposure durations: (a) MEA; (b) Formamide; (c) Acetamide.

Although there is some revocation at 96 h, it is still less than in the control group. Overall, MEA demonstrates a dose-dependent decrease in the microalgal protein. Fig. 4(b) shows the protein content of the microalgae exposed to various concentrations of formamide (0 mg/L to 1 mg/L) over different time intervals. It is observed that formamide affects algal protein in both short- and long-term exposure. The protein content of *Chlorella pyrenoidosa* decreases with increasing formamide (Fig. 4(b)) concentration during all exposure durations. The protein level in the control group (0 mg/L formamide) remains consistently high at 120.84 µg/mL after 0.5 h and 141.51 µg/mL after 48 h of exposure. The protein content of algae decreases to 70–90 µg/mL at a 0.4 mg/L dose and to 60–85 µg/mL at 1 mg/L formamide.

It is evident that protein levels are comparatively greater in the control and decline in response to shorter exposure to the amines (0.5–24 h or 48 h), but improve over a period of longer duration exposure. In the control group, the microalgal protein concentration is quite high, being 118–120 µg/mL at 0.5 h and 72 h. In the presence of different doses of the amine, the pigment concentration fell (40–85 µg/mL). However, the variations were not very specific across different doses and times. Acetamide (Fig. 4(c)) shows less depressing effect on the algal protein as compared to the other two amines. At lower doses of acetamide, protein concentration is little affected, but falls at 0.4–0.6 mg/L, and recovers at higher doses, particularly after 48–72 h of exposure.

The decline in protein content of *Chlorella pyrenoidosa* upon exposure to MEA, formamide, and acetamide suggests that these amines may have induced metabolic stress, inhibiting protein synthesis. Upon exposure to stress, *Pseudochlorella pringsheimii* exhibited varied metabolic responses, including changes in protein synthesis and differential protein expression across growth phases. Impacts on metabolism, antioxidant enzyme activities, cellular recovery mechanisms, oxidative stress tolerance, and ion homeostasis have also been reported upon exposure to different stresses [58]. This study supports the low protein concentration observed at 0.1–0.4 mg/L of MEA, formamide, and acetamide, suggesting that amine toxicity may have interfered with essential metabolic pathways. The partial recovery of protein content following prolonged exposure (72–96 h) suggests an adaptive mechanism in microalgae under a stressful environment [59]. This explains why algae initially reduce protein levels by a significant amount, followed by a recovery period, suggesting compensatory synthesis or the activation of a stress response. The nitrogen source plays a crucial role in determining the protein content of *Chlorella* species; different nitrogen substrates can alter protein accumulation, leading to reduced protein levels [60]. The reduced protein content in *Chlorella pyrenoidosa* suggests that the alga may not have effectively assimilated nitrogen from MEA. The potential nutritional benefits should have been outweighed by the toxic effects of these amines, particularly with formamide. In the case of acetamide, improved protein levels were observed at higher concentrations or during prolonged exposure. This may be due to the metabolic compatibility of acetamide as an organic nitrogen source, as well as environmental conditions and nitrogen availability that can enhance protein content [61, 62].

The two-way ANOVA results for protein levels under MEA, acetamide, and formamide treatments demonstrated strong and statistically significant effects of exposure time, concentration, and their interaction across all three chemicals. In MEA, both time ($F = 18.80$, $p < 0.001$) and concentration ($F = 52.02$, $p < 0.001$) significantly affected protein levels, along with a significant time × concentration interaction ($F = 5.98$, $p < 0.001$). Tukey HSD showed that protein levels at 72 h and 96 h were significantly higher than those at earlier exposures (0.5–48 h). At the same time, the untreated control (0 mg/mL) differed significantly from all treatment concentrations ($p < 0.001$), with multiple mid-dose comparisons (e.g., 0.10 vs 0.40, 0.20 vs 0.60, 0.10 vs 1.00) also showing significant differences, supporting a complex, gradual, dose-dependent trend. Acetamide treatment likewise showed significant effects of time ($p < 0.001$), concentration ($p < 0.001$), and their interaction ($p < 0.001$), with Tukey indicating that protein levels at 72 h and 96 h were significantly higher than early periods and that the untreated control exhibited much higher protein than all treated doses, reflecting a strong multi-level dose response. Formamide treatment produced similar outcomes, with time ($p < 0.001$), concentration ($p < 0.001$), and time × concentration interaction ($p < 0.001$) all significantly influencing protein levels; Tukey HSD revealed a clear rise in protein from 0.5 h to 72–96 h, where early times (0.5–24 h) formed statistically similar groups but were significantly lower than later exposures. Concentration-wise, the control showed the highest protein levels and differed significantly from all treatment doses ($p < 0.001$), while several mid-range concentrations (0.40–0.80 mg/mL) formed closely related groups. Overall, the combined ANOVA and Tukey analyses confirm that MEA, acetamide, and formamide all induce strong, statistically reliable time- and dose-dependent modulation of protein content.

V. Conclusions

The present research shows that both the concentration and the time of exposure to MEA, acetamide, and formamide affect the growth (biomass) and metabolism (chlorophyll and protein) of the freshwater microalga *Chlorella pyrenoidosa*.

The amines had quite different effects on algal physiology; formamide consistently demonstrated the strongest inhibitory effects. Early exposure periods (0.5–24 h) were dominated by acute stress responses across all amines; however, the middle phases (48–72 h) showed distinct recovery patterns, with MEA exhibiting stimulatory effects and acetamide seemingly initiating enzymatic adaptation. Even in relatively hazardous situations, prolonged exposure (96 h) demonstrated impressive potential for physiological adaptation, with partial recovery. Critical periods in algal responses were identified by temporal analysis. Since this temporal variability emphasises the importance of exposure time in toxicity evaluation, short-term studies may not fully reflect the adaptive capability of microalgal systems. Future research should explore the underlying mechanisms of these amines, particularly in aquatic environments where these solvents might accumulate. This study is essential before the environmental impact of the upcoming carbon capture facilities can be accurately assessed.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualisation, N.C.G., A.K. and Dipesh; Methodology, Dipesh, R.T. and E.S.; Software and data analysis, Dipesh and R.T.; Validation and formal experimentation, Dipesh and E.S.; Investigation, N.C.G., A.K., Dipesh, R.T., and E.S.; Resources, N.C.G. and Dipesh; Data curation, Dipesh, R.T., and E.S.; Writing-draft preparation, Dipesh and R.T.; Writing-review and editing, N.C.G., A.K., R.T., Dipesh and E.S.; Supervision, N.C.G. and A.K. All authors had approved the final version.

Funding

This project is funded through the ACT program (Accelerating CCS Technologies), ACT 3 Project No. 327341. Financial contributions made by the Research Council of Norway (RCN), Ministerie van Economische Zaken en Klimaat, the Netherlands, Department for Business, Energy & Industrial Strategy (BEIS) UK, Forschungszentrum Jülich GmbH, Projektträger Jülich (FZJ/PtJ) Germany, Department of Energy (DOE) USA, and Department of Science and Technology (DST) India are gratefully acknowledged.

Acknowledgment

The authors thank the National Collection of Industrial Microorganisms (NCIM), Pune, India, for providing the *Chlorella pyrenoidosa* culture used in this study and acknowledge support from GGS Indraprastha University, Delhi, India, through the FRGS (letter no. F.35(1)(1)/2025/RDC/2975/39).

References

[1] IEA (International Energy Agency). (2021). Net zero by 2050. [Online]. Available: https://www.iea.org/reports/net-zero-by-2050

[2] IPCC, "IPCC fifth assessment report—synthesis report," IPPC, Rome, Italy, 2014.

[3] N. C. Gupta, R. Tanwar, A. Kaushik, R. Singh, A. K. Patra, P. Sar, and P. Khakharia, "Perspectives on CCUS deployment on large scale in India: Insights for low carbon pathways," *Carbon Capture Science & Technology*, vol. 12, 100195, 2024.

[4] S. A. Mazari, P. Alaba, and I. M. Saeed, "Formation and elimination of nitrosamines and nitramines in freshwaters involved in post-combustion carbon capture process," *Journal of Environmental Chemical Engineering*, vol. 7, no. 3, 103111, 2019.

[5] V. Vishal, D. Chandra, U. Singh, and Y. Verma, "Understanding initial opportunities and key challenges for CCUS deployment in India at scale," *Resources, Conservation and Recycling*, vol. 175, 105829, 2021.

[6] P. R. Yaashikaa, P. S. Kumar, A. Saravanan, S. Karishma, and G. Rangasamy, "A biotechnological roadmap for decarbonization systems combined into bioenergy production: Prelude of environmental life-cycle assessment," *Chemosphere*, vol. 329, 138670, 2023.

[7] B. Sreenivasulu, I. Vyas, B. M. Kamani, I. Sreedhar, and K. V. Raghavan, "Effect of composition, synthesis protocol and pellet size of cost effective adsorbents doped with flyash on carbon capture," *International Journal of Environmental Science and Development*, vol. 7, no. 11, 801, 2016.

[8] A. I. Osman, M. Hefny, M. I. A. Abdel Maksoud, A. M. Elgarahy, and D. W. Rooney, "Recent advances in carbon capture storage and utilisation technologies: A review," *Environmental Chemistry Letters*, vol. 19, no. 2, 2021.

[9] D. Bostick, T. Stoffregen, and S. Rigby, "Final techno-economic analysis of 550 MWe supercritical PC power plant CO_2 capture with Linde-BASF advanced PPC technology," Linde LLC, Murray Hill, NJ, United State, 2017.

[10] F. Raganati and P. Ammendola, "CO_2 post-combustion capture: A critical review of current technologies and future directions," *Energy and Fuels*, vol. *38, no.* 15, pp. 13858–13905 2024.

[11] G. T. Rochelle, "Air pollution impacts of amine scrubbing for CO_2 capture," *Carbon Capture Science and Technology*, vol. 11, 100192, Jan. 2024.

[12] A. Rusin and K. Stolecka, "An analysis of hazards caused by emissions of amines from carbon dioxide capture installations," *Polish Journal of Environmental Studies*, vol. 25, no. 3, pp. 909–916, 2016.

[13] H. Lepaumier, E. F. da Silva, A. Einbu, A. Grimstvedt, J. N. Knudsen, K. H. F. Zahlsen, and H. F. Svendsen, "Comparison of MEA degradation in pilot-scale with lab-scale experiments," *Energy Procedia*, vol. 4, pp. 1652–1659, 2011.

[14] D. Botheju, P. Glarborg, and L. A. Tokheim, "NOx reduction using amine reclaimer wastes (ARW) generated in post combustion CO_2 capture," *International Journal of Greenhouse Gas Control*, vol. 10, pp. 33–45, 2012.

[15] US EPA, "In-depth studies on health and environmental impacts of selected water pollutants," Contract No. 68-01-4646, U.S. EPA, Duluth, MN, 1978.

[16] B. Thitakamol, A. Veawab, and A. Aroonwilas, "Environmental impacts of absorption-based CO_2 capture unit for post-combustion treatment of flue gas from coal-fired power plant," *International Journal of Greenhouse Gas Control*, vol. 1, pp. 318–342, 2007.

[17] M. A. Lewis, L. Wolfe, D. Klemm, G. Jordan, J. Lazorchak, and W. Thoeny, "Algae: Sensitive indicators of toxic compounds," *Environmental Toxicology and Chemistry*, vol. 10, no. 8, pp. 1463–1473, 2019.

[18] S. Nigam, M. P. Rai, and R. Sharma, "Effect of nitrogen on growth and lipid content of Chlorella pyrenoidosa," *American Journal of Biochemistry and Biotechnology*, vol. 7, no. 3, pp. 124–129, 2011.

[19] S. Y. W. Chai, L. H. Ngu, and B. S. How, "Review of carbon capture absorbents for CO_2 utilization," *Greenhouse Gases: Science and Technology*, vol. 12, no. 3, pp. 394–427, 2022.

[20] M. K. Mondal, H. K. Balsora, and P. Varshney, "Progress and trends in CO_2 capture/separation technologies: A review," *Energy*, vol. 46, no. 1, pp. 431–441, 2012.

[21] T. Sema, T. Kiattiniracharа, P. N. Ranong, P. Posoknistakul, and R. Jiraratananon, "Density, viscosity, and physical CO_2 diffusivity of novel formulated solvent N-methyl-4-piperidinol and 2-amino-2-methyl-1-propanol for carbon capture," *International Journal of Environmental Science and Development*, vol. 11, no. 10, pp. 483–487, 2020.

[22] F. Meng, Y. Meng, T. Ju, S. Han, L. Lin, and J. Jiang, "Research progress of aqueous amine solution for CO_2 capture: A review," *Renewable and Sustainable Energy Reviews*, vol. 168, 112902, 2022.

[23] C. J. Nielsen, H. Herrmann, and C. Weller, "Atmospheric chemistry and environmental impact of the use of amines in carbon capture and storage (CCS)," *Chemical Society Reviews*, vol. 41, no. 19, pp. 6684–6704, 2012.

[24] E. Gjernes, L. I. Helgesen, and Y. Maree, "Health and environmental impact of amine based post combustion CO_2 capture," *Energy Procedia*, vol. 37, pp. 735–742, 2013.

[25] P. A. Aarrestad and J. O. Gjershaug, "Effects on terrestrial vegetation, soil and fauna of amines and possible degradation products relevant for CO_2 capture. A review," NILU, 2009.
[26] C. Coutris, A. L. Macken, A. R. Collins, N. El Yamani, and S. J. Brooks, "Marine ecotoxicity of nitramines, transformation products of amine-based carbon capture technology," *Science of the Total Environment*, vol. 527, pp. 211–219, 2015.
[27] D. Liu, L. Qin, H. Zeng, Y. Liang, Y. Liang, Y. Chen, and W. Chen, "Ecotoxicological risk assessment of N-nitrosamines to Selenastrum capricornutum in surface waters: Insights into toxicity mechanisms and environmental implications," *Ecotoxicology and Environmental Safety*, vol. 296, 118179, 2025.
[28] N. Gupta, A. Kaushik, R. Singh, A. K. Patra, P. Sar, and P. Khakharia, "Assessment of the impact of various amines on micro- and macro-organisms and their potential biodegradability in the ecosystem," *Sustainable Operation of Post-Combustion Capture Plants (SCOPE)*, 2023.
[29] K. Huang, H. Huang, X. Huang, A. Lao, Z. Zheng, and H. Wu, "Combined toxic effects and mechanisms of chloroacetic acid and N-nitrosodimethylamine on submerged macrophytes," *Water*, vol. 16, no. 18, 2689, 2024.
[30] M. Mitra, T. W. Box, J. S. Gilpin *et al.*, "Isolation and characterization of a novel bacterial strain from a Tris-Acetate-Phosphate agar medium plate of the green micro-alga Chlamydomonas reinhardtii that can utilize common environmental pollutants as a carbon source," *F1000Research*, vol. 9, 656, 2020.
[31] R. Dai, D. Wang, R. Zhu, X. Yang, N. Jiao, L. Sun, and K. Wang, "Thermal-tolerant potential of ordinary Chlorella pyrenoidosa and the promotion of cell harvesting by heterotrophic cultivation at high temperature," *Frontiers in Bioengineering and Biotechnology*, vol. 10, 1072942, 2022.
[32] S. Yin, W. Jin, T. Xi, X. Zhou *et al.*, "Factors affect the oxygen production of Chlorella pyrenoidosa in a bacterial-algal symbiotic system: Light intensity, temperature, pH and static magnetic field," *Process Safety and Environmental Protection*, vol. 184, pp. 492–501, 2024.
[33] Y. Maltsev, K. Maltseva, M. Kulikovskiy, and S. Maltseva, "Influence of light conditions on microalgae growth and content of lipids, carotenoids, and fatty acid composition," *Biology*, vol. 10, no. 10, 1060, 2021.
[34] A. Sánchez-Bayo, V. Morales, R. Rodríguez, G. Vicente, and L. F. Bautista, "Cultivation of microalgae and cyanobacteria: Effect of operating conditions on growth and biomass composition," *Molecules*, vol. 25, no. 12, 2834, 2020.
[35] U. N. Nwankwo and O. K. Agwa, "Temperature and photoperiods optimization for the cultivation of Chlorella vulgaris for growth and biomass production from cassava wastes," *Int. J. Sci. Res. Arch.*, vol. 11, no. 1, pp. 2469–2476, 2024.
[36] A. E. Poste, M. Grung, and R. F. Wright, "Amines and amine-related compounds in surface waters: A review of sources, concentrations and aquatic toxicity," *Science of the Total Environment*, vol. 481, pp. 274–279, 2014.
[37] F. Kasai, N. Takamura, and S. Hatakeyama, "Effects of simetryne on growth of various freshwater algal taxa," *Environmental Pollution*, vol. 79, no. 1, pp. 77–83, 1993.
[38] B. Ma, Y. Chen, H. Hao, M. Wu, B. Wang, H. Lv, and G. Zhang, "Influence of ultrasonic field on microcystins produced by bloom-forming algae," *Colloids and Surfaces B: Biointerfaces*, vol. 41, no. 2-3, pp. 197–201, 2005.
[39] M. Sharma, N. Rani, A. Kamra, A. Kaushik, and K. Bala, "Growth, exopolymer production and metal bioremoval by Nostoc punctiforme in Na and Cr (VI) spiked medium," *Journal of Environmental Research and Development*, vol. 4, no. 2, 2009.
[40] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *Journal of Biological Chemistry*, vol. 193, pp. 265–275, 1951.
[41] G. M. da Rosa, M. G. de Morais, and J. A. V. Costa, "Green alga cultivation with monoethanolamine: Evaluation of CO_2 fixation and macromolecule production," *Bioresource Technology*, vol. 261, pp. 206–212, 2018.
[42] W. Choi, G. Kim, and K. Lee, "Influence of the CO_2 absorbent monoethanolamine on growth and carbon fixation by the green alga Scenedesmus sp.," *Bioresource Technology*, vol. 120, pp. 295–299, 2012.
[43] R. D. Blake and S. G. Delcourt, "Thermodynamic effects of formamide on DNA stability," *Nucleic Acids Research*, vol. 24, no. 11, pp. 2095–2103, 1996.
[44] L. S. Schwardmann, T. Wu, A. K. Dransfeld, S. N. Lindner, and V. F. Wendisch, "Formamide-based production of amines by metabolically engineering Corynebacterium glutamicum," *Applied Microbiology and Biotechnology*, vol. 107, no. 13, pp. 4245–4260, 2023.
[45] R. Singh, R. Shahul, V. Kumar, A. K. Yadav, and P. K. Mehta, "Microbial amidases: Characterization, advances and biotechnological applications," *Biotechnology Notes*, vol. 6, pp. 44–58, 2025.
[46] B. Palenik and S. E. Henson, "The use of amides and other organic nitrogen sources by the phytoplankton Emiliania huxleyi," *Limnology and Oceanography*, vol. 42, no. 7, pp. 1544–1551, 1997.
[47] A. Poste, M. Grung, and R. F. Wright, "Amines in surface waters: A survey of Norwegian lakes," Report. NIVA, Oslo, 2012.
[48] E. Agathokleous, "The rise and fall of photosynthesis: Hormetic dose response in plants," *Journal of Forestry Research*, vol. 32, no. 2, pp. 889–898, 2021.
[49] E. J. Calabrese and M. P. Mattson, "Hormesis provides a generalized quantitative estimate of biological plasticity," *Journal of Cell Communication and Signaling*, vol. 13, no. 4, pp. 317–321, 2019.
[50] R. Mittler, "ROS are good," *Trends in Plant Science*, vol. 22, no. 1, pp. 11–19, 2016.
[51] P. Sharma, A. B. Jha, R. S. Dubey, and M. Pessarakli, "Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions," *Journal of Botany*, vol. 2012, 217037, 2012.
[52] L. S. Schwardmann, L. Benninghaus, S. N. Lindner *et al.*, "Prospects of formamide as nitrogen source in biotechnological production processes," *Applied Microbiology and Biotechnology*, vol. 108, 105, 2024.
[53] S. He, Z. Hao, D. Wang, Y. Guo, H. Wu, A. Ali, and J. Shi, "Formamide deionization accelerates the somatic embryogenesis of Cunninghamia lanceolata," *Forest Systems*, vol. 30, no. 3, 8, 2021.
[54] A. Koletti, D. Skliros, I. Dervisi, A. Roussis, and E. Flemetakis, "Oxidative stress responses in microalgae: Modern insights into an old topic," *Applied Microbiology*, vol. 5, no. 2, 37, 2025.
[55] Z. Chen and J. Q. Xiong, "Recovery mechanism of a microalgal species, Chlorella sp. from toxicity of doxylamine: Physiological and biochemical changes, and transcriptomics," *Journal of Hazardous Materials*, vol. 474, 134752, 2024.
[56] W. M. Brück, E. Alfonso, M. Rienth, and W. Andlauer, "Heat stress resistance in Chlorella vulgaris enhanced by hydrolyzed whey proteins," *Agronomy*, vol. 14, no. 12, 2854, 2024.
[57] W. Guermazi, S. Masmoudi, N. A. Trabelsi, S. Gammoudi, H. Ayadi, A. Morant-Manceau, and G. N. Hotos, "Physiological and biochemical responses in microalgae Dunaliella salina, Cylindrotheca closterium and Phormidium versicolor NCC466 exposed to high salinity and irradiation," *Life*, vol. 13, no. 2, 313, 2023.
[58] M. M. Ismaiel, M. D. Piercey-Normore, and C. Rampitsch, "Biochemical and proteomic response of the freshwater green alga Pseudochlorella pringsheimii to iron and salinity stressors," *BMC Plant Biology*, vol. 24, no. 1, 42, 2024.
[59] M. Klin, F. Pniewski, and A. Latała, "Growth phase-dependent biochemical composition of green microalgae: Theoretical considerations for biogas production," *Bioresource Technology*, vol. 303, 122875, 2020.
[60] F. A. Ansari, H. Hassan, K. M. Gani, I. Rawat, S. K. Gupta, and F. Bux, "Influence of nitrogen sources on growth and biochemical composition of Chlorella sorokiniana," *Biomass and Bioenergy*, vol. 197, 107832, 2025.
[61] J. A. Prates, "Unlocking the functional and nutritional potential of microalgae proteins in food systems: A narrative review," *Foods*, vol. 14, no. 9, 1524, 2025.
[62] Z. H. Koochi, K. G. Jahromi, G. Kavoosi, and A. Ramezanian, "Fortification of Chlorella vulgaris with citrus peel amino acid for improvement biomass and protein quality," *Biotechnology Reports*, vol. 39, e00806, 2023.