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Original Article

Impact of Elevated Ammonia Concentration on *Scenedesmus dimorphus* Growth in a Flat-Plate Photobioreactor

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ABSTRACT

Animal feeding operations (AFOs) are significant sources of airborne pollutants, particularly ammonia (NH_3), which pose considerable environmental and health risks. In response to these challenges, photobioreactor (PBR) systems utilizing microalgae have emerged as a promising solution. These systems can effectively absorb and metabolize pollutants such as NH_3 and carbon dioxide (CO_2), thereby improving air quality while simultaneously producing valuable biomass. The present study specifically investigated the effects of elevated NH_3 concentrations on algal growth within PBRs. Ammonium chloride (NH_4Cl) was employed to simulate NH_3 concentrations typical of animal housing, specifically at a loading rate of 50 ppm (78 mg L^{-1} d^{-1} NH_4Cl). Over a 21-day experimental period, control tanks containing standard Bold's Basal Medium (BBM) were compared against those with NH_3 exposure. Results indicated that while normalized cell concentrations were highest in control tanks (1.79±0.09, $p<0.01$), the dry biomass was significantly greater in tanks subjected to the 50 ppm NH_3 loading rate (1.34±0.02, $p<0.01$). These findings suggest that microalgae possess a remarkable capacity to adapt to high NH_3 levels, highlighting their potential role in emission mitigation and sustainable biofuel production. The integration of PBR systems utilizing microalgae represents a viable strategy for addressing the environmental and health challenges posed by AFOs. By effectively utilizing pollutants such as NH_3, these systems not only enhance air quality but also contribute to the development of sustainable biofuels, thus supporting broader environmental sustainability goals.

Key words: Microalgae, mitigation, animal barns, emission

Yüksek Amonyak Konsantrasyonunun Düz Panel Fotobiyoreaktörde Scenedesmus dimorphus Gelişimi Üzerindeki Etkisi

ÖZ

Yoğun hayvancılık faaliyetleri, amonyak (NH_3) da dahil olmak üzere havayla taşınan önemli kirleticiler üreterek çevre ve sağlık riskleri oluşturmaktadır. Fotobiyoreaktör (PBR) sistemleri, NH_3 ve CO_2 gibi kirleticileri absorbe etmek ve metabolize etmek, hava kalitesini artırmak ve değerli biyokütle üretmek için mikroalgleri kullanan umut verici bir çözüm olarak ortaya çıkmıştır. Bu çalışma, hayvan barınaklarında tipik olarak görülen yüksek amonyak konsantrasyonlarını azaltmak için yüksek NH_3 konsantrasyonlarının PBR'lerde alg büyümesi üzerindeki etkisini araştırmaktadır. Amonyum klorür (NH_4Cl), NH_3 yerine kullanılmış ve nitrat içermeyen BBM ortamı hayvan barınaklarında tipik olarak görülen 50 ppm (78 mg L^{-1} d^{-1} NH_4Cl) NH_3 konsantrasyonunu simüle etmiştir. Her deney 21 gün boyunca normal BBM (0,25 g L^{-1} $NaNO_3$) içeren kontrol tanklarında gerçekleştirilmiştir. Normalize edilmiş hücre konsantrasyonları NH3 içermeyen PBR tanklarında en yüksek (1.79±0.09, $p<0.01$), ancak kuru biyokütle 50 ppm NH_3 yükleme oranına sahip tanklarda daha yüksekti (1.34±0.02, $p<0.01$). Hücre konsantrasyonları 50 ppm NH_3 ile azalırken, kuru biyokütle NH3 içermeyen tanklara kıyasla artmıştır. Sonuçlar, mikroalglerin emisyonları azaltma, yetiştirme stratejilerini optimize etme ve sürdürülebilir biyoyakıt üretimini destekleme potansiyelini ortaya koymakta ve PBR'lerin çevresel etkileri azaltma ve atık kaynakları geri dönüştürmedeki rolünü vurgulamaktadır.

Anahtar kelimeler: Mikroalg, azaltım, hayvan barınakları, emisyon

INTRODUCTION

The increasing concentration of air pollutants in agricultural operations, particularly in animal houses, poses significant environmental and health challenges. Intensive animal husbandry operations generate substantial amounts of airborne pollutants, including ammonia (NH_3), particulate matter (PM), and greenhouse gases, which can adversely affect air quality and contribute to climate change (Guo et al., 2022; Tschofen et al., 2019). Among these pollutants, ammonia (NH_3) is particularly concerned due to its ability to penetrate deep into the respiratory system, posing health risks not only to animals but also to surrounding communities (Xi et al., 2022). As such, innovative strategies are required to mitigate these emissions effectively.

Photobioreactor systems (PBRs) have emerged as a promising technology for addressing air pollution in animal housing environments. These systems utilize microalgae, which can absorb and metabolize various pollutants, including ammonia and carbon dioxide, thereby improving air quality (Uguz et al., 2022; Julianto, 2024). The integration of photobioreactors into animal housing can facilitate the biological treatment of waste gases, converting harmful emissions into biomass that can be further utilized for biofuel production or as animal feed (Uguz and Sozcu, 2024; Dumont, 2018). Research has shown that microalgae such as Scenedesmus species can thrive in controlled environments, effectively utilizing nutrients from waste streams while simultaneously reducing airborne pollutants (Uguz et al., 2022; Sun et al., 2014; Zhang et al., 2014). Moreover, the design and operational parameters of photobioreactors can be optimized to enhance their pollutant removal efficiency. Factors such as light intensity, nutrient concentration, and airflow rates play critical roles in maximizing algal growth and pollutant uptake (Abdel-Baset, 2024; Thành et al., 2016). This innovative approach not only contributes to cleaner air but also promotes sustainable agricultural practices by recycling waste products into valuable resources (Tschofen et al., 2019).

The flat-plate photobioreactors has garnered gained attention due to its potential applications in biofuel production and environmental sustainability. The growth of S. dimorphus is influenced by various factors, including nutrient availability, light intensity, and environmental conditions. Among these factors, ammonia concentration, which is often prevalent in animal housing environments, poses a unique challenge and opportunity for optimizing algal growth. Ammonia can serve as a nitrogen source, which is essential for algal growth, but excessive concentrations can lead to toxicity and inhibit growth (Zhang et al., 2014). Flat-plate photobioreactors are particularly advantageous for cultivating microalgae due to their efficient light utilization and ease of temperature control (Zhang et al., 2012). These systems allow for the precise manipulation of growth conditions, enabling researchers to explore the effects of varying ammonia concentrations on the growth kinetics of *S. dimorphus.* Previous studies have demonstrated that the growth rates of microalgae can be significantly affected by the concentration of nitrogen sources, including ammonia, highlighting the need for careful optimization in photobioreactor designs. Moreover, the interaction between light intensity and nutrient availability, including ammonia, is critical in maximizing biomass productivity and lipid accumulation, which are vital for biofuel applications (Gris et al., 2013; Koller et al., 2016). Research into the effects of ammonia on S. dimorphus growth is essential for understanding how to leverage wastewater and agricultural runoff as nutrient sources in algal cultivation. Such studies not only contribute to the development of sustainable biofuel production methods but also address environmental concerns related to nutrient pollution (Zhang et al., 2014).

This study aimed to investigate the mitigation potential of microalgae grown in a flat-plate photobioreactor to reduce high ammonia concentrations from livestock houses. By examining the impact of high ammonia levels typical of animal houses on the growth of Scenedesmus dimorphus (S. dimorphus) in flat-plate photobioreactors, this research seeks to provide insights that could enhance algal cultivation strategies and promote the use of waste resources for pollutant reduction in animal houses.

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MATERIALS AND METHODS

Algal Cultivation

The microalgae strain (*S.dimorphus*-UTEX 1237) obtained from the UTEX Culture Collection of Algae (Texas University, USA) was used due to its high growth rate and effective NH_3 removal capabilities (Uguz et al., 2022; Kang et al., 2014). *S. dimorphus* was cultivated in a 1 L Erlenmeyer flasks containing 200 mL of Bold's basal medium (BBM), with the pH adjusted to 6.8–7.0 using 0.5M HCl or NaOH. The composition of the BBM can be found in Uguz et al. (2022). The prepared BBM was sterilized at 121°C for 20 minutes. Algae were initially grown in 1-L Erlenmeyer flasks containing 100 mL BBM and doubled weekly, then transferred to 15 L flat plate PBRs (constructed as given by Uguz et al. (2022), Figure 1). During the experiments, the PBR tanks were continuously aerated with air (~350 ppm CO2) at 0.5 L min-1 per PBR volume. Air flowmeters (Cole-Parmer, USA) adjusted the volumetric flow rate.

Flat-Plate Photobioreactor Setup

PBR tanks were constructed from clear acrylic sheets, with the dimensions of 35 cm in height, 50 cm in length, and 10 cm in width. CO_2-enriched air was supplied through a difusser at the bottom of each PBR. Flow meters with needle valves controlled the airflow, adjusted to meet specific air requirements. Each PBR received 7.5 L min^{-1} (0.5 vvm) of aerated air. Illumination was provided by two cool white fluorescent lamps (40 W, 122 cm long, 2100 Lumens) providing 60–70 µmol m^{-2} s^{-1}. Environmental conditions, including pH, temperature, water level (maintained with deionized water), aeration, and light intensity, were monitored throughout the experiments. The pH was adjusted daily to remain between 6.8–7.0 using 0.5 M HCl or 0.5 M NaOH. The room temperature was kept between 23–25°C during the 21-day experimental period. The temperature and pH were measured with a digital pH meter (Hanna Instrument, HI98128). Algae were cultivated under these conditions for 21 days, with daily cell counts measured using hemocytometers under an optical microscope. Figure 1 shows the flat-plate PBR tanks running in the laboratory.

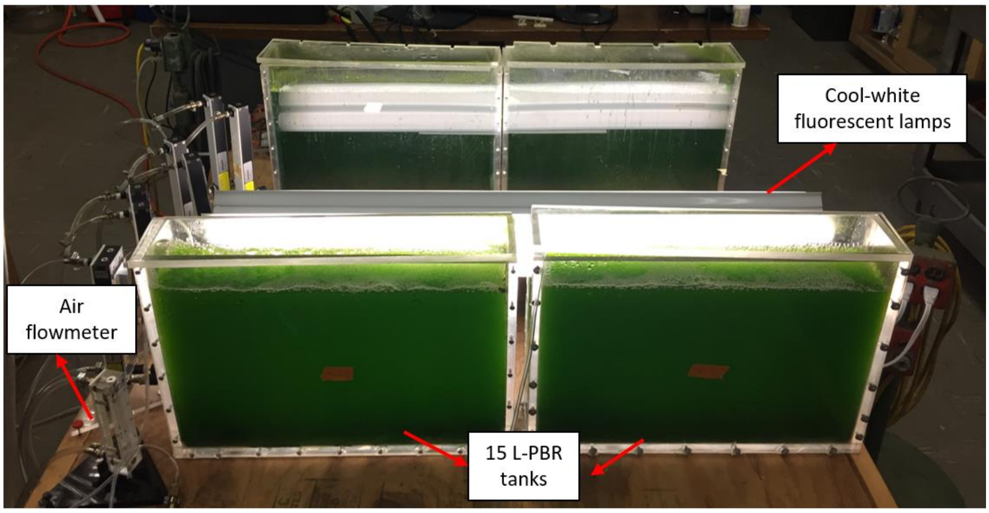

Figure 1. Control and treatment PBRs running in the experiment

Experimental Prodecure

Ammonium chloride (NH_4Cl) was used as a substitute for NH_3 gas found in animal barn air due to NH_3's high solubility and tendency to adsorb onto surfaces, making precise control challenging (Kang et al., 2014; Uguz et al., 2022). This approach aligns with established methods for studying algal responses to ammonia stress under reproducible laboratory conditions. The nitrate-free BBM medium, making NH_4Cl the sole nitrogen source, was added daily to simulate NH_3 concentration of 50 ppm (78 mg $L^{-1}d^{-1}$ NH_4Cl mass equivalent on daily bases). This concentration (50 ppm) represents maximum NH_3 levels in typical animal houses. Ammonia (NH_3) concentrations in animal housing facilities can vary widely depending on livestock type, housing system, and environmental conditions. Typically, levels range from 0 to 40 ppm, but poor ventilation can cause concentrations to exceed 60 ppm (Kim et al., 2021; Tang et al., 2019). Each experiments were run for 21-days with three replicates each, with controls set up for comparison. Control tanks were cultivated with regular BBM (containing 0.25 gL^{-1} $NaNO_3$).

Determination of Growth Parameters

Cell concentrations were determinated using a hemocytometer and an optical microscope (Olympus, Olympus Corp, Tokyo, Japan). The specific growth rate (µ, d^{-1}) was calculated using the Eq. 1:

$$\mu = \frac{\ln N_t - \ln N_0}{t - t_0} \quad (1)$$

where N_0 and Nt are the algae cell concentrations (cell mL^{-1}) at times t_0 and t, respectively (Jiang et al., 2013; Toledo-Cervantes et al., 2013). The dry biomass concentration was determined by vacuum-filtering 25 mL of an algal sample and weighing the filter after drying it in a vacuum oven at 80 °C for 3 hours, following established protocols for dry biomass measurement (g L^{-1}) (Uguz et al., 2022). The algal dry cell weight was calculated by dividing the dry biomass concentration by the cell count of the same sample, then normalizing it to the initial dry cell weight. The biomass weight was determined using the formula provided in Eq. 2 (Uguz et al., 2024).

$$Dry\ biomass\ conc.(gL^{-1}) = \frac{Final\ weight\ (g) - Initial\ weight\ (g)}{Sample\ volume\ (25\ mL)} \times 1000\ (mL/L) \quad (2)$$

NH_4Cl mass equivalent on a daily basis (mg $L^{-1}d^{-1}$) was calculated as:

$$M_{eq}(NH4Cl) = M_{NH3} \times (M_{m(NH4Cl)}/M_{m\ (NH3)} \quad (3)$$

Where $M_{eq}(NH4Cl)$ is the NH_4Cl mass equivalent ($gL^{-1}d^{-1}$), $M_{m(NH4Cl)}$ is the molecular weight of NH_4Cl (53.5 g mol^{-1}), $M_{m\ (NH3)}$ is the molecular weight of NH3 (17 g mol^{-1}), M_{NH3} is the NH_3 loading on daily basis in the influent gas ($gL^{-1}d^{-1}$) and calculation as:

$$M_{NH3} = C_{gas} \times \rho_{gas} \times Q_{PBR} \times (\frac{M_{m(NH3)}}{M_{m\ (air)}}) \quad (4)$$

where C_{gas} is NH3 concentration in the influent air (ppm), ρ_{gas} is the gas density at 25° and 1 atm (0.6943 kg m^{-3}), Q_{PBR} is the airflow rate at each PBR ($m^3\ L^{-1}\ d^{-1}$), and M_m(air) is the molecular weight of air (29 g mol^{-1}).

Statistical Analysis

The results were presented as mean ± standard deviation. Analysis of Variance (ANOVA) and t-tests were conducted to compare the cell and dry biomass concentrations under different test conditions. 95% confidence level was chosen to assess significance. Software package JMP (version 13.0) was used for the statistical analysis.

RESULTS AND DISCUSSION

PH in the PBR Tanks

The pH of algal culture media is a critical factor influencing algal growth, nutrient absorption, and overall biomass productivity. Optimal pH levels are essential for maximizing photosynthesis and metabolic processes in microalgae. Research indicates that most algal species thrive within a pH range of approximately 6 to 8 (Chaudhuri, 2020). Deviations from this range can lead to significant physiological stress, inhibiting enzyme activity and photosynthetic efficiency, ultimately resulting in reduced biomass production (Ambat et al., 2019; Rana, 2024). The relationship between pH and nutrient availability is particularly noteworthy. For instance, the solubility of essential nutrients such as nitrates and phosphates is pH-dependent, which directly affects algal growth (Rana, 2024; James et al., 2013). At higher pH levels, the availability of carbon dioxide decreases, which can limit photosynthesis and growth rates (Singh, 2017). Conversely, low pH levels can lead to the inhibition of enzyme activity critical for nutrient uptake and metabolic processes (Ambat et al., 2019; Rana, 2024). Therefore, maintaining an optimal pH is vital for ensuring that microalgae can efficiently utilize available nutrients.

The pH of the each PBR tank was monitored daily and adjusted to 7 using 0.5M HCl or 0.5M NaOH. The pH was influenced by dissolved CO_2 in the air and NH_3 provided by NH_4Cl, as well as their uptake by algal cells. An initial pH increase in the control PBRs on Day 1 was due to the photosynthetic uptake of CO_2 by algae. Daily adjustments maintained a stable pH in the control group. In experimental groups receiving NH_4Cl, pH ranged from 5.1 to 7.1 throughout the experiment, with significant drops observed after Day 15 due to high NH_4Cl concentrations. Subsequent algal uptake and daily adjustments helped stabilize the pH. Figure 2 shows the pH change throughout of the 21-day experiment.

The dynamics of pH changes in algal cultures supplemented with NH_4Cl are influenced by several factors, including the initial concentration of ammonium, the specific algal species, and the overall metabolic activity of the culture. For instance, studies indicate that as algal cells grow and fix carbon dioxide, they can initially increase the pH of the medium through the consumption of CO_2, which is a weak acid (Roopnarain et al., 2014). However, when ammonium is added, the subsequent uptake can lead to a net decrease in pH, particularly if the concentration of ammonium is high (Pahl et al., 2012). This interplay between ammonium uptake and pH dynamics is crucial for optimizing algal cultivation conditions, as excessively low pH levels can lead to detrimental effects such as reduced enzyme activity and impaired nutrient absorption (Zhao et al., 2015; Ayre et al., 2017). The addition of ammonium chloride to algal culture media has a profound effect on pH, which in turn influences algal growth and productivity. The complex interactions between ammonium uptake, pH dynamics, and environmental factors necessitate careful monitoring and management to optimize algal cultivation conditions.

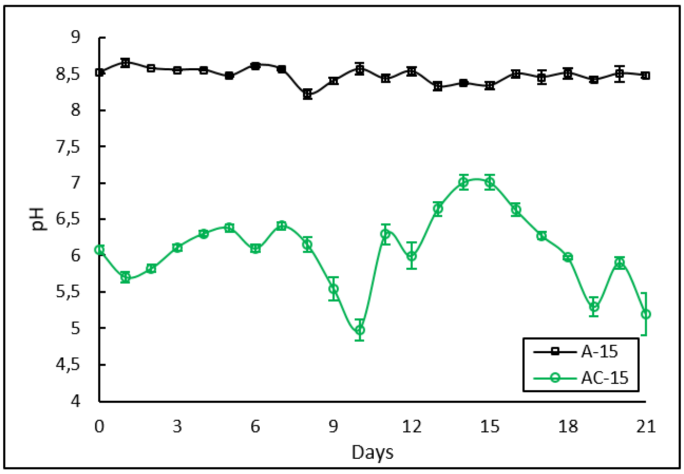

Figure 2. PH changes during the 21-day experiment (AC-15: PBR tanks fed with NH4Cl and A-15 control tanks)

Cell and Dry Biomass Concentration

Ammonia (NH_3) concentrations in animal housing can reach significant levels, varying by species and housing conditions. In animal production facilities, ammonia concentrations typically range from 0 to 40 ppm, with reports of levels exceeding 60 ppm under poor ventilation (Kim et al., 2021; Tang et al., 2019). Specifically, ammonia can reach up to 35 mg/m³ (about 25 ppm) in winter due to reduced ventilation (Tang et al., 2019). In poultry houses, particularly in broiler and layer systems, ammonia levels often range between 25 and 30 ppm, sometimes exceeding 50 ppm in extreme cases (Yi et al., 2016; Wei et al., 2015). For years, our team has worked on integrating photobioreactor systems into animal production to reduce ammonia and carbon dioxide. Given that ammonia levels in production can reach 50-60 ppm, this study investigates the impact of 50 ppm ammonia (worst-case scenario) on microalgae growth in photobioreactors. In this study, to investigate the effect of high NH_3 concentrations on algal growth in PBRs, microalgae in A-15 PBR tanks were grown with standard BBM, while AC-15 PBR tanks were fed daily with 78 mg L^{-1} d^{-1} NH_4Cl, equivalent to a 50 ppm NH_3 loading rate. Figure 2 shows the normalized cell and dry biomass concentration, and normalized dry cell weight of *S.dimorphus* grown under different culture conditions**.** Normalized algal cell number concentrations was the highest (1.79±0.09, $p<0.01$) in the PBR tanks without NH_3. However, the dry biomass concentration was higher (1.34±0.02, $p<0.01$) in the PBR tanks fed with 50 ppm NH_3 gas loading rate. Figure 3 shows the normalized cell and dry biomass growth curves of *S.dimorphus* for 21-day experiment

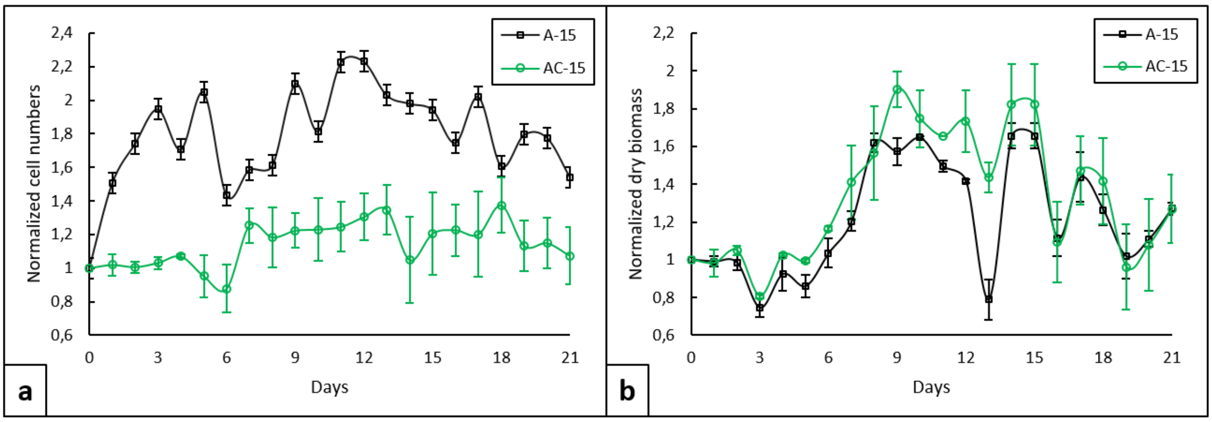

Figure 3. Normalized cell and dry biomass numbers of S.dimorphus for 21-day experiment

Experiments showed a decrease in algal cell concentrations towards 50 ppm NH_3, but dry biomass concentration was increased at 50 ppm NH_3 loading rate compared to NH_3-free PBR tanks (Figure 4). Ammonium ions (NH_4^+) are a preferred nitrogen source for many algal species, as they can be assimilated more readily than other nitrogen forms, such as nitrates or urea. However, the effects of varying NH_3 concentrations on algal growth are complex and can lead to both beneficial and detrimental outcomes. At optimal concentrations, ammonium can significantly enhance algal growth by providing essential nitrogen for the synthesis of proteins, nucleic acids, and chlorophyll, which are vital for cellular functions and photosynthesis (Vasileva et al., 2020). For instance, studies have shown that moderate levels of ammonium can promote the growth of species like *Chlorella vulgaris* and *Chlamydomonas reinhardtii*, leading to increased biomass and lipid production (Liu et al., 2017; Ayre et al., 2017). The presence of free ammonia (NH_3), which can form at higher pH levels, poses additional risks, as it is more toxic to algal cells than ammonium ions (Qian et al., 2023). The relationship between ammonia concentration and algal growth is also influenced by environmental factors such as pH, temperature, and the presence of other nutrients. For instance, maintaining a balanced pH is essential, as a drop in pH due to high ammonium levels can further exacerbate growth inhibition (Scherholz & Curtis, 2013). Additionally, the interaction between ammonium and other nitrogen sources can affect overall growth dynamics; some algal species may exhibit preferences for certain nitrogen forms, which can influence their growth rates under varying ammonia conditions (Vasileva et al., 2020).

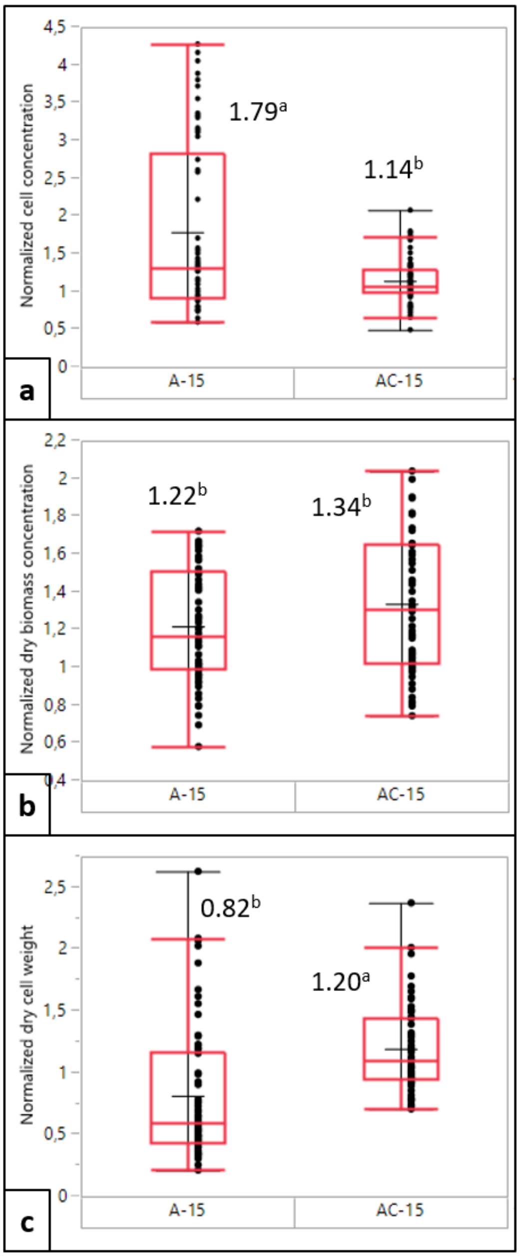

Figure 4. Normalized cell and dry biomass concentration, and normalized dry cell weight of *S.dimorphus* grown under different culture conditions

The NH_3 concentration in algal culture media significantly influences the dry biomass concentration of algal species, with varying effects depending on the ammonia levels present. Ammonium ions (NH_4^+) are a preferred nitrogen source for many microalgae, promoting growth and biomass accumulation when supplied in optimal concentrations. However, the relationship between ammonia concentration and algal biomass is complex, as both low and high concentrations can lead to distinct outcomes in biomass production. At moderate ammonia concentrations, algal growth is generally enhanced due to the efficient assimilation of nitrogen, which is crucial for protein synthesis and cellular metabolism. For instance, studies have shown that algal species such as *Chlorella protothecoides* exhibit increased biomass production when supplied with adequate ammonium levels, as nitrogen is a key component of *chlorophyll* and other cellular structures (Shabana et al., 2019; Ambat et al., 2019).

The ability of algae to uptake NH_3 efficiently can lead to significant increases in dry biomass concentrations, particularly in nutrient-rich environments (Wrede et al., 2018). Conversely, excessive NH_3 concentrations can have detrimental effects on algal growth and biomass accumulation. High levels of ammonium can lead to toxicity, primarily due to the formation of free ammonia (NH_3) at elevated pH levels, which is harmful to algal cells (Zhu et al., 2020; Palkar et al., 2020). Research indicates that when NH_3 concentrations exceed certain thresholds, algal growth can be inhibited, resulting in lower dry biomass concentrations. This toxicity can manifest as decreased photosynthetic efficiency, impaired metabolic functions, and ultimately reduced biomass accumulation (Wu et al., 2020).

The influence of ammonia concentration on algal biomass is also mediated by environmental factors such as temperature, light intensity, and the presence of other nutrients. For instance, Wrede et al. (2018) observed that seasonal variations in ammonia concentrations correlated with changes in algal biomass, with higher NH_3 levels in winter associated with lower biomass production (Wrede et al., 2018). This suggests that while NH_3 is essential for algal growth, its effectiveness as a nutrient source is contingent upon optimal environmental conditions.

CONCLUSION

The findings in this study indicate that microalgae, specifically *Scenedesmus dimorphus*, can efficiently utilize elevated levels of ammonia typical of animal housing environments, converting these pollutants into valuable biomass. Optimal growth conditions, including controlled pH, temperature, and nutrient levels, are essential for maximizing algal growth and pollutant uptake. The research highlights that flat-plate PBRs are particularly advantageous due to their efficient light utilization and ease of temperature control, enabling the precise manipulation of growth conditions to enhance pollutant removal efficiency. The study also emphasizes the importance of optimizing PBR operational parameters, such as light intensity, nutrient concentration, and airflow rates, to maximize biomass productivity. The ability of microalgae to thrive under varying ammonia concentrations makes them a sustainable solution for reducing environmental pollutants in agricultural settings. Moreover, the generated biomass can be further utilized for biofuel production or as an alternative protein source in animal feed, contributing to a circular economy. Overall, this research underscores the potential of PBR systems as a sustainable technology for improving air quality in animal housing environments while supporting bioresource recovery. Future research should focus on scaling up these systems, refining operational strategies, and exploring the long-term impacts of using microalgae-based feed supplements to enhance the economic feasibility and environmental benefits of PBR integration in animal agriculture.

For Türkiye’s livestock sector, where intensive poultry, dairy, and cattle operations are prevalent, integrating PBR technology could address critical environmental and economic challenges. Türkiye’s livestock houses often face elevated ammonia levels (frequently exceeding 30 ppm), particularly in densely populated regions like the Aegean and Central Anatolia, where ventilation is limited during winter months. The high ammonia emissions not only pose health risks to animals and workers but also contribute to soil and water eutrophication. Microalgae-based PBRs could be deployed in these settings to:

- Reduce Ammonia Emissions: By absorbing NH_3 directly from barn air, PBRs could complement existing ventilation systems, especially in confined poultry and dairy facilities where ammonia concentrations peak.
- Utilize Local Resources: Türkiye’s abundant sunlight and moderate climate favor outdoor or greenhouse-integrated PBRs, reducing energy costs for algal cultivation.
- Generate Co-Products: The harvested biomass could supplement animal feed (e.g., for poultry or aquaculture), addressing Türkiye’s reliance on imported feed ingredients while valorizing waste.
- Comply with Regulations: As Türkiye strengthens environmental policies (e.g., alignment with EU IPPC directives), PBRs could help farms meet emission limits sustainably.

However, challenges such as initial investment costs, maintenance expertise, and seasonal temperature fluctuations must be addressed through pilot projects and government incentives. Future research should focus on optimizing PBR designs for Türkiye’s regional climates (e.g., insulating systems for eastern Anatolia’s harsh winters) and quantifying long-term economic benefits for farmers. By aligning PBR technology with Türkiye’s livestock characteristics—such as small-to-medium farm sizes and high ammonia-producing poultry operations—this approach could advance both environmental sustainability and circular bioeconomy goals.

Main significant results of the study should be presented in a clear and concise way and should be numbered.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Author Contributions

1st Seyit Uguz : Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; writing— original draft; writing—review and editing.

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