The Effect of pH on Growth and Carbon Sequestration Efficiency of *Chlorella vulgaris*

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ABSTRACT

The increasing urgency of climate change has driven the need for innovative carbon mitigation strategies. Microalgae, particularly C.*vulgaris*, have garnered attention for their potential to capture atmospheric CO_2 while contributing to ecological sustainability. This study investigates the optimization of carbon sequestration potential of *C. vulgaris* under varying pH levels (3.0 to 11.0) using BBM medium. Growth performance was assessed by measuring optical density (OD750) and biomass accumulation, while carbon sequestration was quantified by calculating CO_2 equivalent fixation over a 14-day culture period. The results reveal that C. vulgaris exhibited maximum carbon fixation at pH 9, demonstrating its adaptability to optimized environmental conditions. These findings emphasize the potential of C. vulgaris as an efficient, scalable, and eco-friendly carbon sink, positioning it as a viable candidate for integration into urban green infrastructure and climate mitigation strategies. This research provides valuable insights for policy frameworks aiming to incorporate biological solutions into the fight against climate change.

Keywords: C. vulgaris, carbon sequestration, microalgae, climate mitigation, pH, biomass, phycotechnology.

INTRODUCTION

The escalating levels of atmospheric carbon dioxide (CO_2) are a critical driver of global warming, necessitating sustainable mitigation strategies. Among nature-based solutions, microalgae—particularly *Chlorella vulgaris*—have emerged as promising biological agents for carbon sequestration due to their rapid growth rates (doubling in <24 h under optimal conditions) and high photosynthetic efficiency [1]. *C. vulgaris* has been successfully cultivated in diverse systems, including membrane photobioreactors and bubble column reactors, with CO_2 fixation rates exceeding 1.5 g L^{-1} day^{-1} when optimized for light, nutrients, and pH [2, 3].

pH is a pivotal factor influencing algal physiology, affecting nutrient solubility, enzyme activity, and membrane stability [4]. While prior studies have explored *C. vulgaris* growth under varying pH, a systematic evaluation of pH's dual impact on biomass yield and carbon sequestration efficiency is lacking. This study bridges that gap by assessing *C. vulgaris* performance across a broad pH range (3.0–11.0). Our findings aim to inform scalable cultivation systems for carbon capture, aligning with global efforts to deploy algae-based climate solutions.

MATERIALS AND METHODS

A. Culture Conditions

C. vulgaris were bought from Algatech Sdn. Bhd. and Universiti Sains Malaysia (USM), Pulau Pinang. The microalgae were subcultured in Bold Basal's Medium (BBM) at 24 °C, under 16:8 of light and dark cycle. It is triplicate in BBM medium (pH 6.8, adjusted with 0.1 M KOH/HCl) under sterile conditions. Cultures were maintained at 24 ± 0.5°C (mean ± SD) under continuous illumination (1800 lux, cool white LEDs) with thrice-daily manual agitation to ensure homogeneity [5].

B. Growth Metrics

An initial concentration of 1 × 10^5 cells/mL of *C. vulgaris* was inoculated into 48-well microplates and cultured for 15 days under controlled conditions. The cultures were maintained at 25 ± 1°C, with a light intensity of 60–70 µmol m^{-2} s^{-1}. Experiments were conducted across varying medium formulations, pH levels, and photoperiods. Growth was monitored daily by measuring optical density at 750 nm (OD_{750}) using a Mettler-Toledo UV7 spectrophotometer. This wavelength is commonly used to estimate algal biomass without interference from chlorophyll or other pigments [2].

C. Carbon Sequestration Calculation

After 14 days of cultivation, the biomass of *C. vulgaris* grown in different media, pH levels, and photoperiods was harvested and measured. The collected samples were freeze-dried for three days to determine dry weight (DW), then stored at -80°C for further analysis [4]. The amount of carbon sequestered was calculated based on the total carbon content in the dry biomass, using a standard conversion factor of 0.427. Total CO_2 sequestration was then estimated by multiplying the carbon content by 3.67, which represents the molecular weight ratio of CO_2 to carbon [11].

Carbon sequestered (C) = D.W. (Cell productivity) × 0.427.

(0.427 is the conversion factor based on six replicates of Dry weight vs carbon values)

CO 2 sequestered = C × 3.67.

where C is carbon sequestered, D.W. is amount of dry weight of cyanobacterium mass and 3.67 is a constant.

RESULT

A. Effect of pH on Growth

The growth performance of C.*vulgaris* was assessed by measuring optical density at 750 nm (OD_{750}) under different pH conditions. As shown in Figure 1, pH had a significant effect on algal growth. At pH 3, growth was nearly absent, with an absorbance value of 0.01, indicating poor cell viability under highly acidic conditions. A slight improvement was observed at pH 5 (absorbance = 0.08), while growth further increased under neutral conditions (pH 7), reaching an absorbance of 0.11. The highest growth was recorded at pH 9, with an absorbance of 0.200, indicating that mildly alkaline conditions favour optimal cell proliferation. However, growth declined sharply at pH 11, where the absorbance dropped to 0.032. This suggests that extreme alkalinity inhibits the photosynthetic efficiency and metabolic activity of *C. vulgaris*.

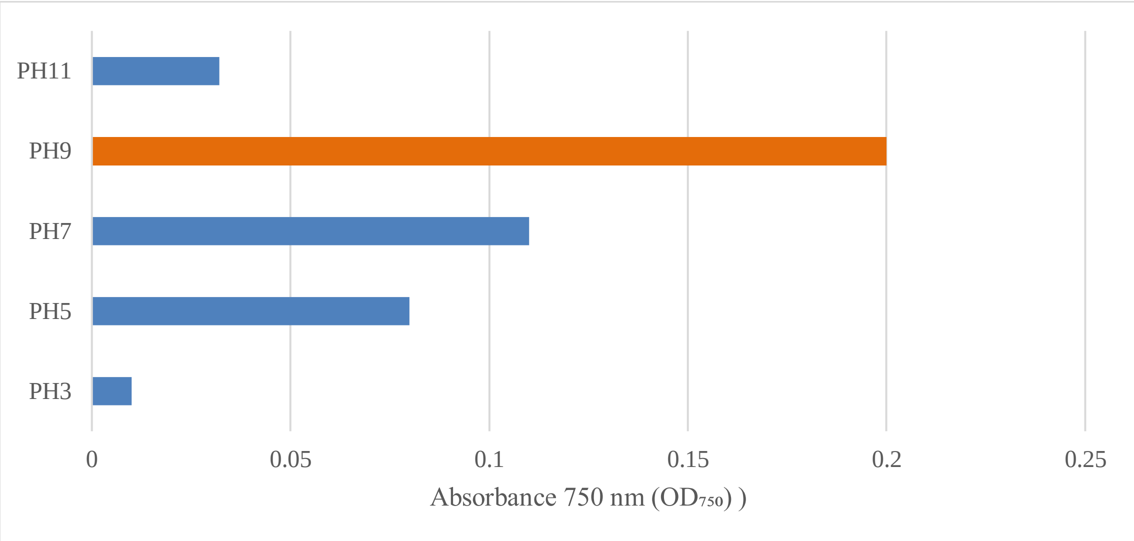

Fig. 1 Optimized condition of the algal growth

B. Effect of of pH on Biomass Production and Carbon Sequestration

In alignment with the growth trends, both biomass production and carbon sequestration rate (CSR) showed marked variation across the pH spectrum (see Figure 1). No measurable biomass or carbon sequestration was observed at pH 3 (0.00 g/L; 0.00 CO_2e). At pH 5, only a minimal biomass yield of 0.048 g/L and CSR of 0.075 CO_2e were obtained. Moderate values were recorded at pH 7, with 0.361 g/L biomass and 0.566 CO_2e. The most favorable results were achieved at pH 9, yielding the highest biomass (0.784 g/L) and carbon sequestration rate (2.851 CO_2e). Interestingly, although pH 11 resulted in a relatively high biomass (0.485 g/L), the CSR was considerably lower (0.760 CO_2e), suggesting diminished carbon fixation efficiency under excessive alkalinity. These findings suggest that pH 9 provides the most favorable environment for maximizing both growth and carbon capture potential in C. vulgaris cultivation.

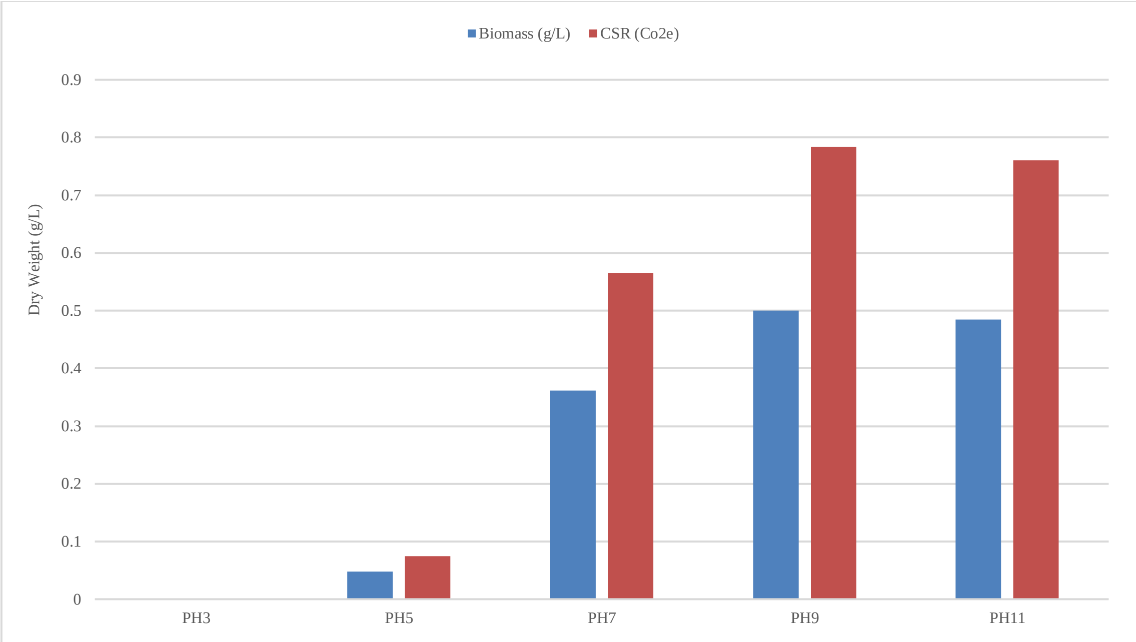

Fig. 2 Effect of pH on Biomass and Carbon Sequestration Rate

DISCUSSION

Some strains of *C. vulgaris* respond differently depending on the pH. For example [5], found that the 3N813A strain, which releases maltose, tends to grow better in lower pH conditions. However, even though it can grow, its photosynthesis performance still drops at pH 5.0, it only reached 76% of its photosynthetic capacity compared to at pH 7.0. This suggests that while the strain can tolerate lower pH, it may shift its energy from growth to stress responses like producing maltose, which reduces carbon available for biomass. Other studies found that a slightly acidic to neutral pH (around 6.3 to 6.8) is often ideal for growth [6]. These conditions help keep enzyme activity stable and allow better nutrient absorption. In contrast, alkaline conditions can be useful for carbon capture. Adding a wood biomass ash (WBA), an alkaline material, to the growth medium not only improved the growth of C. vulgaris but also helped absorb more CO_2 from flue gases. This makes alkaline environments potentially useful in carbon sequestration applications [2].

Interestingly, the ideal pH also depends on what product is being targeted. The highest biomass was produced at pH 8, while the best oil content was found at pH 6. This means that choosing the right pH depends on whether the goal is to produce more biomass or extract valuable bioproducts in mixed cultures, pH also affects competition between species [7]. *C. vulgaris* grows best at pH 9.0 when cultured alone. However, when grown with *Anabaena sp.*, a cyanobacteria, it struggled to compete, especially at higher pH levels [8]. This suggests that *C. vulgaris* may be less efficient in multi-species systems under certain pH conditions. The relationship between pH and growth was further supported by [9] who found that growth picked up after a slow start and peaked around pH 7.0 to 7.5. Similarly, [1] showed that in co-cultures, the algae reduced the pH of the environment over time, indicating their ability to help stabilize conditions during wastewater treatment.

Overall, the findings confirm that pH is a key factor in determining how well grows and captures carbon. While it can survive in a range of pH levels, the most favorable conditions for both growth and carbon sequestration are typically between pH 7 and 9. Understanding and managing pH carefully can help improve algal performance in both lab settings and large-scale environmental applications.

CONCLUSIONS

This study demonstrates that pH significantly influences the growth and carbon sequestration efficiency of *C.vulgaris* with optimal performance observed under mildly alkaline conditions (pH 9), achieving maximum biomass production (0.784 ± 0.042 g L^{-1}) and CO_2 fixation (2.851 ± 0.21 CO_2e). The pronounced inhibition of growth and carbon uptake at extreme pH levels (≤5 or ≥11) highlights the delicate balance required for efficient microalgal cultivation, where alkaline conditions favor bicarbonate utilization while avoiding metabolic stress. While these results validate *C. vulgaris* as a promising candidate for biological carbon capture, practical implementation faces challenges in maintaining stable pH at industrial scales, particularly in open pond systems exposed to environmental variability. Future research should prioritize pilot-scale trials using flue gas as a carbon source to evaluate economic feasibility, alongside exploring sustainable pH stabilization methods such as waste-derived alkaline additives. Additionally, investigating mixed algal consortia or biofilm reactors could enhance system resilience and reduce operational costs. By addressing these scalability challenges, *C. vulgaris*-based carbon sequestration can transition from laboratory validation to practical climate mitigation applications, offering an environmentally sustainable pathway to complement existing carbon reduction strategies. The findings provide both a scientific foundation for optimizing algal cultivation and critical insights for policymakers considering nature-based solutions in carbon neutrality roadmaps.

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