Effects of different zinc concentrations on ammonia nitrogen removal during microalgae cultivation in swine wastewater

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Highlights:

- Concentrations between 1 and 100 mg Zn/L were tested and the dose of 25 mg Zn/L favored algal growth.
- N-NH_4^+ conversion processes such as assimilation and nitrification were negatively affected in treatment with 100 mg Zn/L.
- The dominant process of N-NH_4^+ removal in the control (1 mg Zn/L) and 5 mg Zn/L treatments was possibly the nitrification.
- It is possible to suggest that in the other treatments (10, 25 and 50 mg Zn/L) assimilation was favored

Keywords: Agro-industrial effluent; algal biomass; nitrate.

INTRODUCTION

The cultivation of microalgae in wastewater is attractive due to its ability to produce several metabolic compounds of commercial value, such as carbohydrates, proteins and lipids. At the same time, treatment occurs with the removal of nutrients and the supply of oxygen necessary for the biological oxidation of organic matter. However, all opportunities involving the cultivation of microalgae in wastewater can be compromised due to the presence of toxic compounds present in effluents, such as metals. An example of a production sector that produces effluent with worrying concentrations of metals is swine farming, in which the zinc content range between 12 and 234 mg/L (Zeng et al., 2021).

Zinc is considered a micronutrient for microalgae, so adjusted concentrations can stimulate algal growth. While concentrations above necessary can cause toxicity. This interference in algal development can affect nutrient removal during wastewater treatment (Oliveira et al., 2023). Among the nutrients of greatest concern in swine wastewater, ammonia nitrogen can be highlighted, which can reach levels of 1650 mg/L (Cheng at al., 2019). This content is a significant threat to the environment to confers a risk of eutrophication, to result in oxygen depletion in water that can kill aquatic organisms.

Therefore, the objectives of this study were to evaluate the interference of different concentrations of Zn on the growth of algal biomass and the removal of ammonia nitrogen from swine wastewater (SW). Identifying these effects is essential to propose optimization measures for microalgae biotechnology and contribute to its application, especially in rural areas that face technical, economic, and governance challenges in wastewater management.

METHODOLOGY

This study was developed at the Sanitary and Environmental Engineering Laboratory, at the Federal University of Viçosa (UFV), Brazil. The SW used was collected within an aerated lagoon at the Swine Teaching, Research and Extension Unit at UFV. In this unit, sixty matrices are maintained in the intensive confinement system with a complete production cycle. SW presented the following characteristics: chemical oxygen demand = 162.8 mg/L; ammonia nitrogen ($N\text{-}NH_4^+$) = 78.4 mg/L, nitrate (NO_3^-) = 12.7 mg/L, total phosphorus = 15.0 mg/L and total zinc (Zn) = 1.0 mg/L.

For the cultivation of microalgae, six high-rate ponds (HRPs) were used on a pilot scale with a useful volume of 1 m³. These ponds were made of fiberglass with six-blade steel paddlewheels moved by a 1 hp electric motor that, through a reducer and a frequency inverter, maintains the effluent speed between 0.10 m/s and 0.15 m/s. Each HRP was fed with 0.8 m³ of SW and 0.1 m³ of microalgae inoculum consisting of the mixed biomass previously cultivated in the SW. One of the HRPs was used as a control treatment (without the addition of Zn, it only had the concentration that was present in SW), while the other five had Zn concentrations adjusted to 5, 10, 25, 50, and 100 mg Zn/L with zinc chloride solution.

The HRPs were operated in a batch mode until the decline in chlorophyll-*a* (chl-*a*) concentration that was monitored every three days. The chl-*a* was quantified as described in Oliveira et al. (2023). Cell counting took place in a Neubauer chamber using a light microscope (Olympus Corporation, model

CX40 RF 100) and it was realized for the days when the highest concentrations of chl-*a* were observed. Every three days, pH was measured using a multiparameter probe (Hach model HQ30D/LDO). Furthermore, every 5 days, the content of $N\text{-}NH_4^+$ and NO_3^- was monitored according to APHA (2017). The results were processed using Sigma Plot 12.0 software.

RESULTS AND CONCLUSIONS

The monitoring period varied between 10 days (for HRP with 5 mg Zn/L and the control treatment) and 29 days (for HRP with 100 mg Zn/L), because the growth lag phase was greater according to the Zn concentration increased (Figure 1A). It was noted that treatments with the application of Zn gave a higher number of cells (from 2.73 to 7.43 x 10^6 cells/mL) compared to the control treatment (1.10 x 10^6 cells/mL) (Figure 1B). HRPs with 5, 50 and 100 mg Zn/L showed similar cell count values while the application of 10 and 25 mg Zn/L achieved the best results, with emphasis on the latter which presented 7.43 x 10^6 cells/mL. Similar behavior was reported by Hamed at al (2017), the authors verified an increase in the cell density of *Chlorella sorokiniana*, with values between 10 and 14 x 10^6 cells/mL, when cultivated in synthetic medium enriched with 26.1 and 39.2 mg Zn/L while the application of 65.4 mg Zn/L inhibited growth.

Zinc makes up several enzymes that are important for the development of microalgae, such as alkaline phosphatase, superoxide dismutase, RNA polymerase and carbonic anhydrase (Baścik-Remisiewicz et al., 2009). For the conditions adopted in the present study, it can be suggested that the dose of 25 mg Zn/L was the most favorable condition to meet Zn demands. Lower concentrations were possibly insufficient and higher doses may have inhibited algal growth since Zn can induce the production of reactive oxygen species (Oliveira et al., 2023).

Regarding variations in $N\text{-}NH_4^+$, it is believed that the contribution of volatilization was less significant as the monitored pH ranged from 6.9 to 8.5. Assimilation and nitrification were probably the dominant processes and enabled removals between 93 and 100% in HRPs with up to 50 mg Zn/L (Figure 1C). Still, in the treatment with 100 mg Zn/L, the highest concentrations of $N\text{-}NH_4^+$ can were observed with a value close to the maximum limit for disposal of effluents into water bodies at a national level (20 mg/L) (Brazil, 2011), indicating that assimilation and nitrification were inhibited. This occurrence can be observed when checking variations in NO_3^- concentrations, being that HRP with 100 mg Zn/L showing the lowest concentrations of NO_3^- with levels lower than 13 mg NO_3^-/L even after 25 days of algal cultivation (Figure 1D).

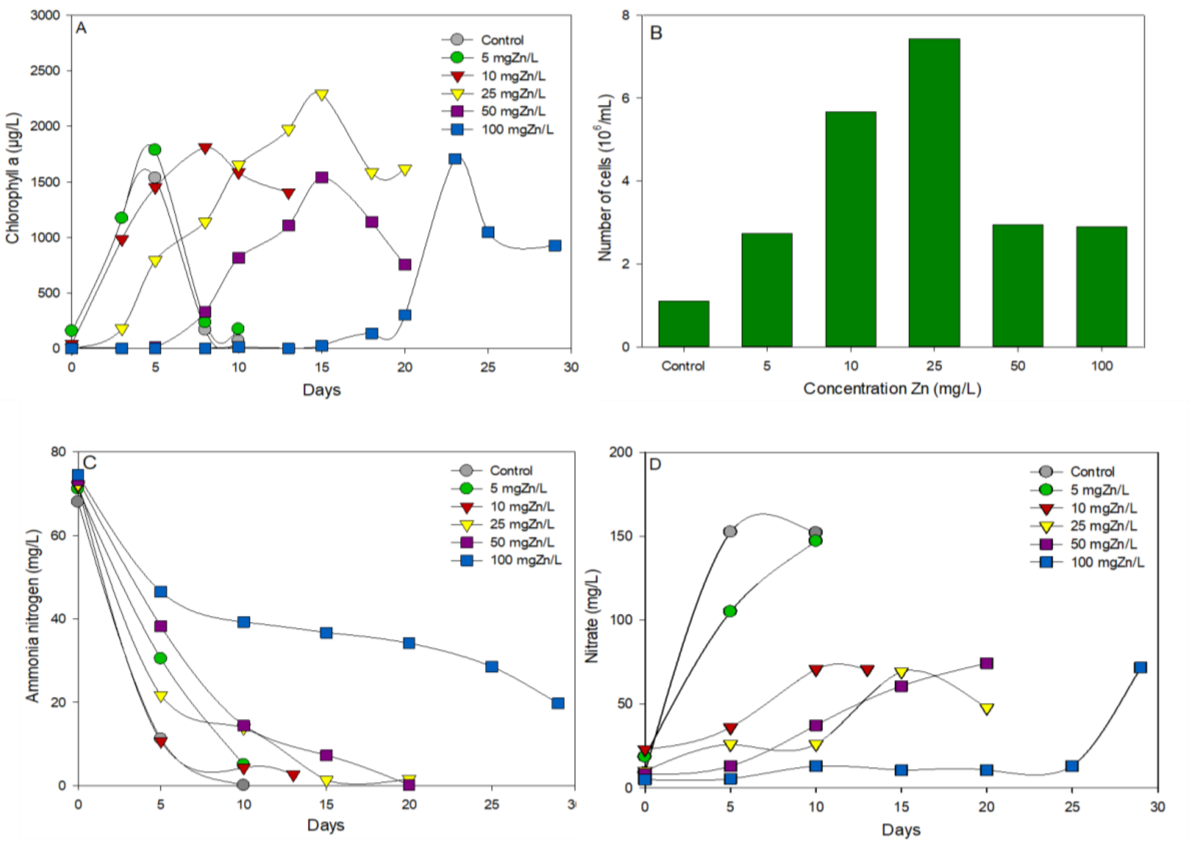

Figure 1: Variation in (A) chlorophyll-*a*, (B) number of cells, (C) ammonia nitrogen concentration, and (D) nitrate quantified during cultivation of microalgae at different concentrations of Zn.

Interestingly, it was observed that the control treatment and the addition of 5 mg Zn/L conferred the highest concentrations of NO_3^- (up to 152.0 mg NO_3^-/L). These treatments possibly provided the most appropriate conditions for the development of nitrifying microorganisms, which is not consistent with the treatment that contributed to the highest cell density of microalgae (25 mg Zn/L). The fact that a diverse microbial community develops in HRPs is reflected in different demands for Zn, therefore, the concentrations of this element can be adjusted to favor the processes of greatest interest.

Similar results reporting the effects of Zn on ammonia nitrogen removal from SW were reported by Li et al (2020) and Zhang et al (2018). Oliveira et al (2023) observed that the main effects of Zn on N conversion in HRAPs during SW treatment were: (i) Zn did not contributed to pH increases and therefore the formation and volatilization of NH3 were not favored and (ii) nitrification proved to be a sensitive process to the presence of Zn being severely compromised as the levels of this metal increased.

ACKNOWLEDGMENTS

This work was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) [Finance Code 001]. Also, the authors gratefully acknowledge the financial of the Civil Engineering Department from the Federal University of Viçosa, the National Council for Scientific and Technological Development (CNPq) [Grant Number: 310319/2020-0] and Minas Gerais Research Support Foundation (FAPEMIG) [Grant Numbers PCE-00449-24, RED-00068-23].

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