KINETIC OF NUTRIENT REMOVAL FROM SUGARCANE VINASSE INTO BIOMASS OF *Phormidium autumnale*

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ABSTRACT

Vinasse is the main wastewater from ethanol production from sugarcane broth, with a characteristic strong odor, dark brown color, low pH, high potassium content and high pollutant potential due to the load of organic matter. In Brazil, technologies applied in the sugarcane processing generate approximately 10 L of vinasse per liter of ethanol. As vinasse is generated at around 100ºC, it is essential to cool it and evaluate the temperature for the microbial growth. Among the species that have gained notoriety in research on effluents is the cyanobacterium *Phormidium autumnale*. Therefore, the objective of this research was to evaluate the kinetics of incorporation and consumption of nitrogen, phosphorus and potassium from sugarcane vinasse by *Phormidium autumnale* at different temperatures. The results indicated maximum nitrogen and phosphorus removal at 25ºC (79 and 85%, respectively). The greatest incorporation of potassium into microalgal biomass was obtained at 35ºC (40% within 24 h), normally reported as the optimum temperature growth for cyanobacteria. Even at the highest temperature evaluated, the one where the least cooling of the vinasse would be necessary, there are removals of phosphorus and potassium that allowed the recycling of these nutrients with the use of the biomass generated for various applications.

Keywords: Vinasse. Microalgae. Nutrient removal. *Phormidium*. Heterotrophic growth.

1 INTRODUCTION

Vinasse is the main wastewater from sugarcane processing, characterized by its strong odor, dark brown color, low pH, high potassium content and polluting potential due to the high load of organic matter. In Brazil, technologies applied in the fermentation-distillation generate approximately 10 L of vinasse per liter of ethanol. As it is estimated that the 2024/25 harvest will lead to a production of around 27 billion liters of ethanol solely from sugarcane, this will represent a generation of approximately 280 billion liters of vinasse [1]. In addition to organic matter and potassium, other nutrients are present in this wastewater and their recovery could represent the obtaining of a product usable for the most diverse purposes, keeping the remaining vinasse for its main current destination, that is, in fertigation of sugarcane.

Microalgae and cyanobacteria have been researched to remove nitrogen and phosphorus from agro-industrial wastewater. Although preferably photosynthetic, some strains of these microorganisms are capable of developing without light source from the consumption of simple organic molecules, which is known as heterotrophic growth. Thus, the generation of microalgal biomass would be combined with the reduction of organic matter and nutrient levels in agro-industrial effluents. However, there are few studies in the literature that address the estimation of kinetic parameters of nitrogen, phosphorus and potassium consumption from vinasse.

The use of vinasse in microalgae growth has been gaining prominence, as they are capable of obtaining high biomass productivity linked to the high accumulation of biomolecules of economic and industrial interest [2,3,4]. Furthermore, it is known that cyanobacteria in particular have the ability to incorporate nutrients into cells during growth under certain temperature and light conditions.

The highest specific maximum growth rates of cyanobacteria, around 0.6 to 0.8 day^{-1}, occur in the ranges of 25 to 35ºC [5]. As it is an effluent from the bottom of the distillation column, vinasse is generated at a high temperature, around 105ºC, and its cooling is mandatory for use in fertigation and/or as microbial cultivation media. In any case, evaluation at temperatures above the ranges indicated for cyanobacteria could make this process viable on an industrial scale, considering the large daily volumes of vinasse generated on a sugarcane processing platform.

As vinasse has a high turbidity, its use as a cultivation medium occurs by strains of microalgae that experience the so-called "heterotrophic metabolism", that is, non-limiting by light. *Phormidium autumnale* is a microalga that has demonstrated notoriety and tolerance to extreme conditions such as use in industrial effluents. It is recognized as a robust species normally found in extreme environments due to its tolerance to high concentrations of nutrients and temperatures. It is a filamentous cyanobacterium, with filaments 3 to 4 µm in diameter, unbranched, with a typical blue-green color and easy aggregation and recovery from the production medium.

The objective of this research was to evaluate the incorporation and consumption kinetics of nitrogen, phosphorus and potassium present in sugarcane vinasse into biomass of cyanobacteria *Phormidium autumnale*.

2 MATERIAL & METHODS

Cyanobacterium *Phormidium autumnale* isolated from the Cuatro Cienegas Desert, Mexico, was kindly provided by the Federal University of Santa Maria (UFSM), maintained and propagated in BG11 [6] at 25ºC and 12-hour light-dark photoperiod.

The experiments were set up without light (heterotrophic conditions) using vinasse collected at a sugarcane processing unit in Araras, São Paulo, Brazil. The cultures set up in triplicate on an orbital shaker with sampling at pre-defined time intervals at 25, 35 and 45ºC during 72 hours.

Microalgae biomass was separated from culture medium by centrifugation (1280 RCF for 15 minutes) and vinasse remaining was characterized in terms of total nitrogen by TOC-LCPN SIMADZU®, phosphorus by colorimetric method, and potassium by flame photometer [7]. The removals of these nutrients and the kinetic consumption constants were calculated from the concentration profiles obtained during the growth of the microalgae.

3 RESULTS & DISCUSSION

Figure 1 shows the nitrogen, phosphorus and potassium profiles of cell-free vinasse at different times. The curves indicate that nutrients are incorporated into the biomass in the first moments, with part of them being released into the cultivation medium at the end of the batches, probably due to cell lysis.

In all cases there is a consumption trend that follows first-order kinetics, that is, fast incorporation in the first hours and dependent on the nutrient concentration. Furthermore, the results indicate a great influence on temperature, with the highest consumption profiles occurring at 25 or 35ºC. According to Visentin et al [4], temperature is the most significant variable in the heterotrophic cultivation of *Phormidium autumnale* in vinasse, mainly influencing the metabolism of microalgae and macromolecular structures of cells [8].

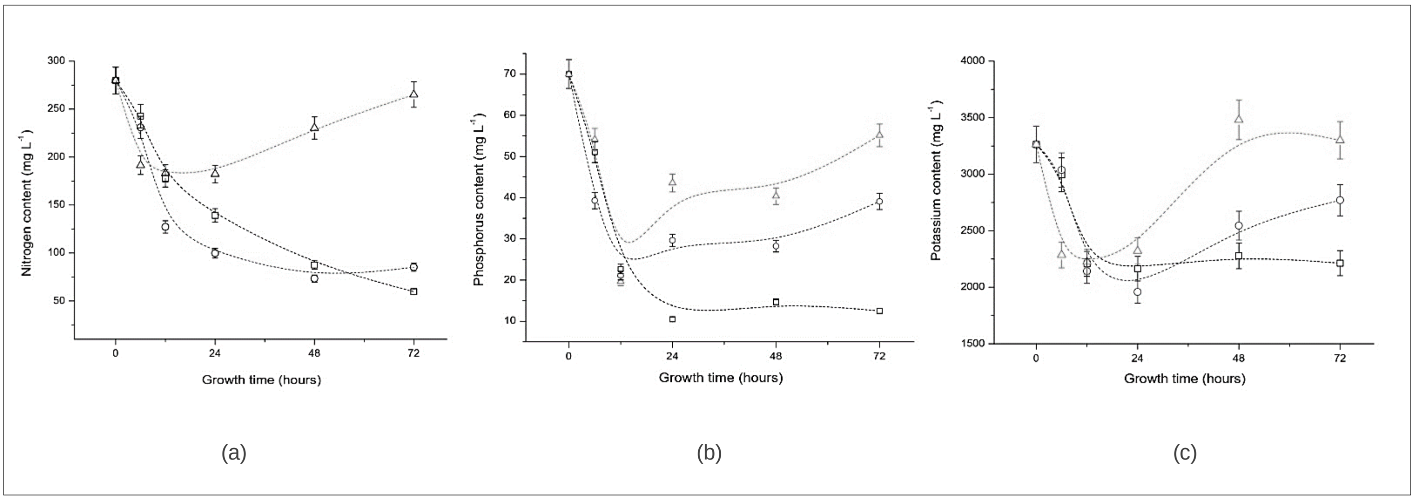

Figure 1 Profiles of nitrogen, phosphorus and potassium from vinasse during cultures of *Phormidium autumnale* at 25 (□), 35 (○) and 45ºC (∆)

As vinasse is generated at high temperatures, above 100ºC, evaluating its use in conditions above 40ºC would facilitate cooling, which is inevitable. According to the results presented in Table 1, even at 45ºC, with as little cooling as possible, it would be possible to use biomass with 35% nitrogen, more than 70% phosphorus and more than 30% potassium. Thus, in addition to minimizing the environmental impact of the large amount of vinasse generated by the sugarcane harvest, the nutrients removed could be recycled in the soil via the application of microalgal biomass.

On the other hand, the highest kinetic constants for nitrogen and phosphorus consumption occur at 25ºC (0.021 and 0.082 h^{-1}, respectively), the condition where growth is maintained for longer. Thus, the nitrogen in the vinasse is gradually consumed, reaching a maximum removal of 79% at the end of the batch, in 72 hours. In the case of phosphorus, the maximum removal of 85% was obtained in 24 hours, a longer time compared to 35 and 45ºC. The nutrient that seems least influenced by temperature is potassium, which presents removals of around 30 to 40% for all conditions, with release into the medium after 24 hours.

This high incorporation of potassium into microalgal cells must be linked to greater growth at this temperature, indicated as the optimal range for biomass production [5]. This is evidenced by the high nitrogen removals at 25 and 35ºC, as nutrient that is directly related to cell growth, so much so that it has a consumption kinetic constant that is an order of magnitude lower for 45ºC.

Table 1 Kinetics parameters for nitrogen, phosphorus and potassium removal by *Phormidium autumnale*

	25ºC	35ºC	45ºC
Kinetic consumption first-order constant for nitrogen (h^{-1})	0.021	0.016	0.0024
Maximum nitrogen removal (%)	79% at 72 h	74% at 48 h	35% at 24 h
Kinetic consumption first-order constant for phosphorus (h^{-1})	0.082	0.10	0.043
Maximum phosphorus removal (%)	85% at 24 h	70% at 12 h	71% at 12 h
Kinetic consumption first-order constant for potassium (h^{-1})	0.033	0.035	0.032
Maximum potassium removal (%)	34% at 24 h	40% at 24 h	32% at 12 h

The results indicate that, regardless of the temperature, it is possible to recycle part of the vinasse nutrients, generating a microalgal biomass that can be used as biofertilizer for various agricultural crops. Thus, in addition to minimizing the polluting impact of vinasse in terms of nitrogen and phosphorus, since it is applied directly to the soil, biomass as a by-product of this product has a commercial value, even after minimal cooling of the wastewater to 45ºC.

4 CONCLUSION

The experimental results showed maximum nitrogen and phosphorus removals at 25ºC, 79 and 85%, respectively. The high incorporation of potassium into microalgal biomass was obtained at 35ºC (40% in 24 h), related to greater cell growth. In any case, even at temperatures above the optimum range, phosphorus and potassium were incorporated, recycling these vinasse nutrients with a reduction in the polluting potential of this wastewater and the feasibility of using microalgal biomass.

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