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Consumption of the Pollutant Load of Mezcal Vinasse by *Stigeoclonium nanum* in Semi–Continuous Operation

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The Vinasse is a mezcal distillation byproduct that contains large amounts of contaminants, which can affect the environment. An alternative for reducing the concentration of organic and inorganic material that Vinasse contains is implementing bioprocesses with semi-continuous operation. This research evaluated *Stigeoclonium nanum* in a photobioreactor flat panel with a capacity of 15 L of operation with a dilution rate of 0.033 d^{-1}(1L/ every two days) of Vinasse, quantifying growth of microalga and reduction of the high concentration of contaminants with daily determinations of photosynthetic pigments, the nitrogen of nitrates, total phosphorus, CDO, total carbohydrates and ammoniacal nitrogen. The results obtained were 143.06mg/L of total production of chlorophylls throughout the experiment, consumption percentages of nitrogen of nitrates, total phosphorus, CDO, and total carbohydrates of 59 %, 87 %, 6 %, and 23 %, respectively. The ammoniacal nitrogen does not present significant consumption, however its concentration is below 10 mg.

1. Introduction

Mezcal is considered an artisanal beverage; its elaboration process begins with harvesting agaves that have a maturation time between 8 and 10 years. At this age, they are cut from the base to obtain the piñas and take them to be cooked. At this stage in the maguey, being at high temperature, hydrolysis is generated, and the starches present are converted into glucose and fructose; then, the fermentation process is finished, in which the sugars in the must are converted into alcohol. Finally, to separate the alcohol from the must, the process is carried out using distillation, where the undesirable solids or liquids present in the raw material are separated, the effluent being called vinasse. For every 1 L of alcohol, approximately 13 to 15 L of vinasse is produced, which contains high amounts of organic load such as alcohol and aldehydes, among others, and also has very high chemical oxygen demand (COD) concentrations, turbidity, and an acidic pH, making it a hazardous waste for the environment because it can damage soil fertility and extinguish aquatic life (Bermeo-Garay et al., 2017). An alternative to reduce the pollutant load of this effluent is to apply bioprocesses in which the use of living organisms makes it possible to reduce the concentration of pollutants. Using biotechnology to reduce the pollutant load in the treatment of wastewater from the food or domestic industry has the advantage that the growth of these photosynthetic microorganisms does not present great difficulties since they have a high tolerance to pollutants, as they can present a mixotrophic metabolism in the presence of nutrients of organic and inorganic origin, generating high biomass production (Manzoni-Maroneze et al., 2021). Microalgae are photosynthetic microorganisms that can present autotrophic, heterotrophic, and mixotrophic metabolism depending on the nutritional environment in which they are found and the light conditions. These characteristics generate scientific interest due to their biotechnological and commercial potential. Microalgae biotechnology

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has different applications, such as food supplements, biofuel, cosmetology, and phytoremediation, where the pollutant load of effluent is used as a nutrient by microalgae in growth kinetics (Franco Martínez et al., 2017; Gómez-Luna, 2007).

Stigeoclonium nanum is a freshwater microalga of colonial structure with branched filaments over 200 µm long; its cells are barrel-shaped or cylindrical (Guiry and Guiry, 2022). Their biomass composition is always a function of the nutrient conditions present in the culture conditions and the photobioreactor's (PBR's) configurations to be employed for higher biomass concentration. Martínez-Roldán et al. (2019) report the use of a flat panel photobioreactor for the cultivation of *S. nanum*. This configuration provides better light distribution to the culture, reaching higher biomass productivity. In semi-continuous mode, the microorganism is kept in an exponential phase until reaching an equilibrium point with growth rate and nutrient consumption. This strategy helps reduce the pollutant load of effluents such as whey or vinasse, causing a reduction of the compounds present in the effluent.

2. Materials and methods

A flat panel FBR with a capacity of 15 L was inoculated with mineral medium BG11 (Blue-Green medium), inoculated with 2.234 ± 0.167 mg/L of total chlorophyll, presenting an autotrophic batch culture during the first seven days. Subsequently, semi-continuous operation of the system was initiated at a dilution rate of 0.033 d^{-1} with replacement every third day, performing growth kinetics for 29 days with daily quantifications of photosynthetic pigments, $N\text{-}NO_3$, $N\text{-}NH_4$, total phosphorus, and total carbohydrates, COD was performed when the system was replaced. Table 1 shows the methodologies used. The system was maintained under a light intensity of 250 µE m^{-2} s^{-1}, 22 ± 3 °C. The initial pH was of 7.4 and the when starting the dilution rate the pH was of 9.7 (this is because the pH of the vinasse was modified by addition of NaOH to a value of 9).

Table 1.- Methodology used

Quantification	Methodologic	Reference
Photosynthetic pigments	Extraction of methanol	Wellburn (1994)
$N\text{-}NO_3$	Quantification spectrophotometric with resorcinol technique	Zhang and Fischer (2006)
$N\text{-}NH_3$	Quantification spectrophotometric with phenol and nitroprusside	Franco Martínez et al. (2017)
Total phosphorus	Quantification spectrophotometric with persulfate digestion	Morris and Gales (1966)
Total carbohydrates	Quantification spectrophotometric with phenol	Dubois et al. (1951)
COD	Quantification spectrophotometric	Rice et al. (2017)

3. Results and discussion

The use of effluent as a source of nutrients for microalgae cultivation seeks to reduce organic matter by taking advantage of the mixotrophic metabolism of the microalgae; in addition, the increase in oxygen condensation and pH by the action of photosynthesis stimulates the aerobic bacteria present in the system. Consequently, the microorganisms present will integrate nitrogen, phosphorus, and some other micronutrients, decreasing the overall pollutant load of the effluent (Franco Martínez et al., 2017).

Characterization of vinasse

The vinasse study used for this research showed high COD and carbohydrate concentrations, reaching 11866.67 ± 1027.54 and 3662.96 ± 1.07 mg/L, respectively. Nitrogen compounds such as $N\text{-}NH_3$ and $N\text{-}NO_3$, as well as total phosphorus, are found in lower proportions, reaching concentrations of 3.02 ± 0.16, 37.47 ± 2.47, and 24.05 ± 2.62 mg/L, respectively, in addition to an acid pH of 3.64. The vinasse has a strong brown color and this changes the transparency of the culturing medium (Figure 1)

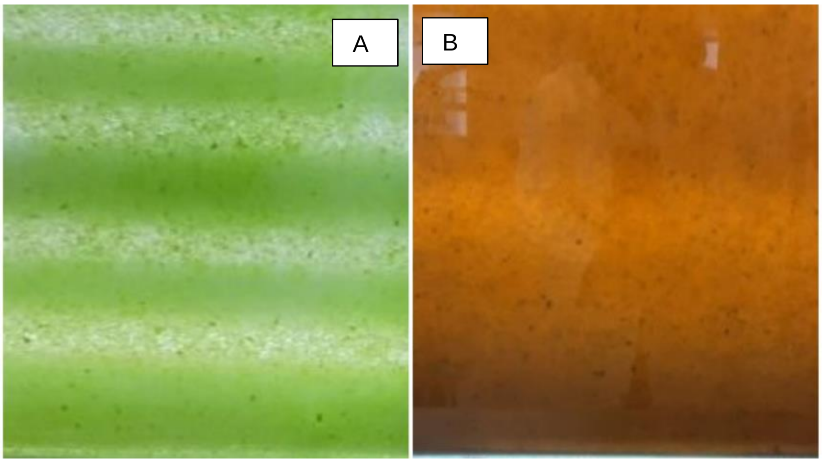

Figure 1.- A) S. nanum inoculated in mineral medium BG11. B) S. nanum in operation semi-continuous with vinasse

Growth of *Stigeoclonium nanum* using vinasse as culturing medium

At the beginning of the semi-continuous mode in the system, the total chlorophylls (Chlt) presented a concentration of 5.14 ± 0.346 mg/L; from time 20, the production of total chlorophylls reached an equilibrium concerning the input and output of nutrients, not exceeding 2.5 mg/L d, ending the experiment with a total production of 146.06 mg Figure 2.

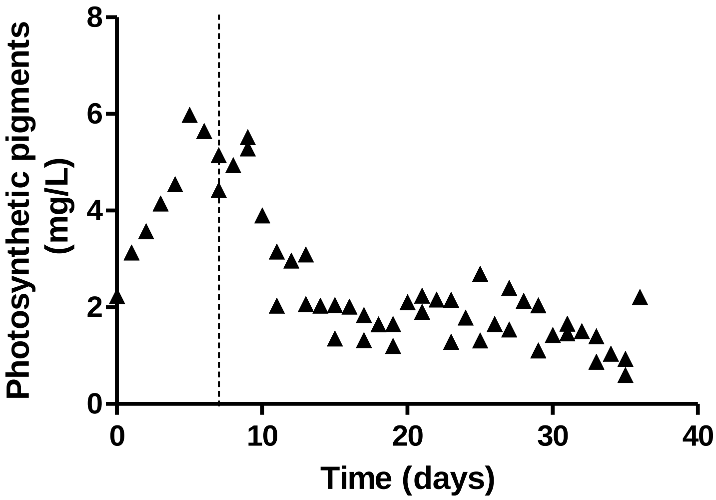

Figure 2.- Total chlorophylls as an indicator of microalgae presence in the system, the dotted line represents the beginning of the semi-continuous mode in the system.

Consumption of organic nutrient

Microalgae, in the presence of vinasse, use organic carbon from carbohydrates to consume it as a nutrient for their growth. This study observed a consumption of 23% during the growth kinetics. Between days 7 and 19, an increase in total carbohydrate concentration was observed. However, from day 20 on, a balance between the rate of nutrient consumption and nutrient input was observed until the end of the operation mode in the Figure 3A system. COD, when related to the amount of biodegradable organic matter, shows a similar behavior to the carbohydrate concentration (Figure 3B); however, COD only shows a consumption of 6 %.

Consumption of inorganic nutrient

Nitrogen sources such as N-NH_3 and N-NO_3, when encountered simultaneously, are consumed differently. At the beginning of the experiment, the concentration of N-NO_3 110.61 ± 5.68 mg/L presenting in the semi-continuous mode, before the first replacement on day seven, a concentration of 81.54 ± 5.11 mg/L obtaining a percentage of consumption during the whole kinetics of 59 % using vinasse as culturing medium result that is similar with what is reported Xin et al. (2010) and Zhuang et al. (2018) that present consumption values of above of 50% using mineral medium as culturing medium. In the case of N-NH_3, given that there is a growth in the concentration of biomass, it would be expected that there would be a consumption, however, the system does not present an obvious decrease in the concentration of N-NH_3 (Figure 4), this could be due to the presence of proteins in the system and their subsequent hydrolysis favor the increase of N-NH_3 which is consistent with report Santana et al. (2017) using vinasse and mineral medium in different microalgae who reported proteins production in similar experiments with determination as N-NO_3, N-NO_2, COD, total phosphorus, N-NH_3.

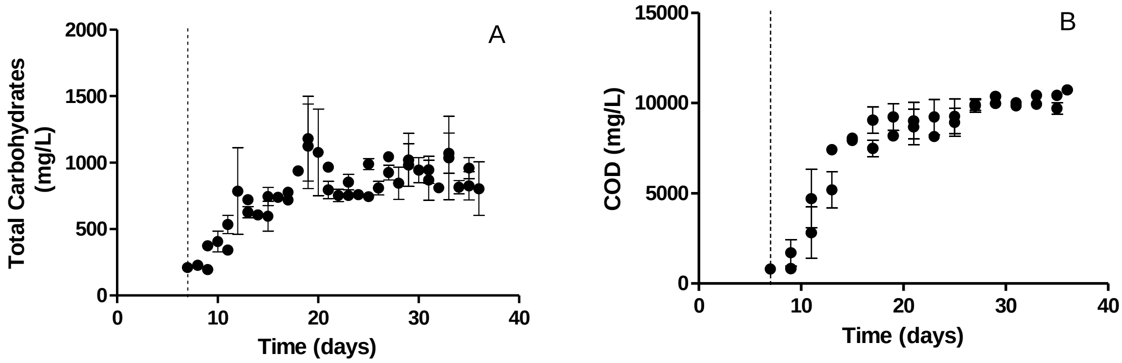

Figure 3.- A Total Carbohydrates and B COD, the dotted line represents the beginning of the semi-continuous mode in the system.

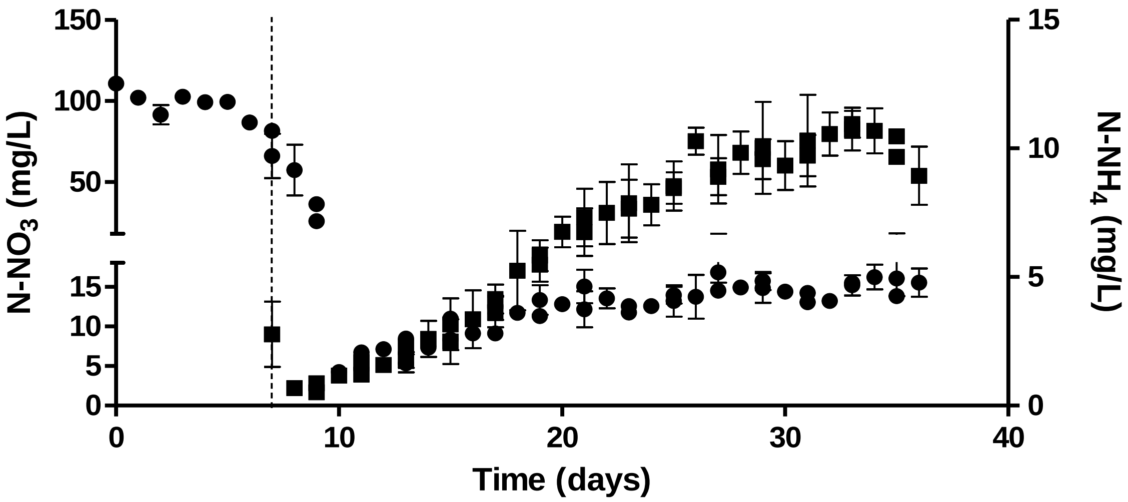

Figure 4.- Consumption of nitrogen sources in the system in semi-continuous mode ● N-NO_3 and ■N-NH_3, the dotted line represents the beginning of the semi-continuous mode in the system.

Total phosphorus does not present high concentrations in the effluents so the microalgae can consume most of the compound. The concentration of total phosphorus (Figure 5) in the autotrophic batch culture presents a concentration of 4.22 mg/L at time zero, reaching a concentration of 2.47 mg/L at time seven before replacement. Concerning this nutrient, a constant balance is not achieved between input and output of the nutrient in the system, because behavior of the balance remains constant during the first 10 days, a period in which the concentration of this nutrient reaches a value between 1 and 2 mg/L. From day 18 onwards, significant increases are observed at the replacement time. However, these increases in concentration stimulate the consumption of phosphorus so that the total balance shows a consumption of 87 % during the entire kinetics.

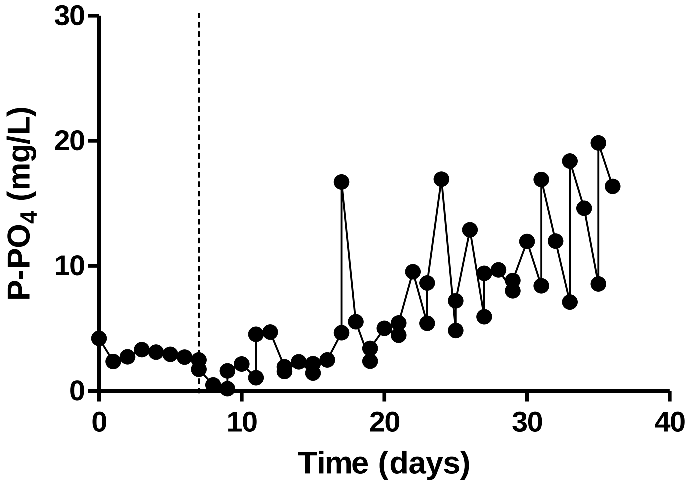

Figure 5.- Total phosphorus intake, the dotted line represents the beginning of the semi-continuous mode in the system.

The system was operated for 29 days, which corresponds to a residence time; according to Levenspiel. (1998), to consider that a system has reached equilibrium, there residence times must pass. However, when using effluent from mezcal production it was not possible since from day 28 changes were observed in the behavior of the system consistent with the beginning of a general collapse; due to this was decided to end the experiment. To extend the operating time of this treatment system, it would be necessary to modify other operating parameters that were not considered in this work.

4. Conclusions

The system's operation favors the tolerance and survival of the microalgae; however, when working with an effluent, higher percentages of consumption of the different compounds present in the stillage were expected. The high concentration of organic matter and the presence of coloration in the effluent did not allow for high efficiencies in the elimination of the pollutant load of the effluent.
To improve the elimination of the pollutant load of the stillage, other strains that present greater tolerance could be used to modify the dilution rate so that the stillage's entry rate decreases, allowing the microalgae to develop better.

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