EFFECTS OF A SUBMERGED AGITATION PUMP ON *Spirulina* sp. GROWTH DYNAMICS AND TRICHOME INTEGRITY

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ABSTRACT

The production of microalgae has been increasing worldwide, with biomass products praised for their proteins, functional carbohydrates, pigments, and antioxidant content, with applications as food ingredients and even the pharmaceutical industry. Cultivating these photosynthetic microorganisms requires special attention to aeration, nutritional demand, and lighting. In some cases, the aeration system provides enough agitation, maintaining homogeneity for light capture in the bioreactor. In large systems, however, aeration is seldom enough, and agitation strategies must be included, such as paddle wheels or pumps. *Spirulina* sp., a staple among microalgal bioproducts, is a filamentous cyanobacterium, with cells united in trichomes with a spiral morphology of different sizes, which can reach more than 100 µm in length. These trichomes can break under excessive shear, such as that expected inside pump rotors. In this work, a commercial submersible pump was used to evaluate the cultivation of *Spirulina* sp. and the viability of its trichomes. The results showed that the type of pump used generated shear on the *Spirulina* sp. trichomes, but the cyanobacterium maintained its growth during the evaluation period. After the experiment, it can be concluded that submersible pumps damage *Spirulina* sp. trichomes and may reduce productivity, but not enough to forbid its use in large-scale cultivation.

Keywords: Spirulina. Trichome. Growth. Shear. Aeration. Agitation.

1. INTRODUCTION

Spirulina sp., a genus of filamentous cyanobacterium belonging to the family *Oscillatoriaceae*, displays a distinctive morphology characterized by regularly coiled, cylindrical, multicellular trichomes in an open left-hand helicoidal shape. The structure of the trichomes, which can reach more than 100 µm, is integral to its functionality and resilience in various environments. *Spirulina* cultures with fractured, small, or linear filaments are more difficult to collect and tend to have lower quality (1). These characteristics also play a significant role in the microorganism's ability to grow in tropical and subtropical waters with high salt content and alkaline pH levels, demonstrating significant adaptability and growth potential under these conditions. Various media, such as Zarrouk's, have been employed to optimize its growth, addressing the need for effective nutrient management for commercial production (2)

Aeration and agitation play crucial roles in the cultivation of *Spirulina* sp., impacting both growth rates and morphological integrity. The proper design of microbiological production systems must consider the hydrodynamic stress tolerance of microorganisms, as excessive stirring and aeration can harm sensitive microorganisms, reducing their growth and metabolism, and causing the release of intracellular substances. These stresses can damage fragile microorganisms, resulting in lower growth rates and metabolic retardation. Therefore, optimizing aeration and agitation parameters is essential to maintaining the viability and productivity of *Spirulina* cultures (3).

Compressors and pumps are usually employed to achieve aeration and agitation. For laboratory and pilot processes, conventional compressors usually agitate *and* aerate cultures, but these do not promote enough homogenization of cultures at larger scales. Submersible pumps can be used to agitate and homogenize the culture, thus improving the culture illumination, as well as the availability of nutrients dissolved in the culture medium. However, these devices can negatively affect the culture due to the high shear the pumps exert when moving the liquid, which can deform the culture cells to the point of causing cause cell lysis; this effect is known as shear stress (3,4). This study aims to evaluate the effect of the shear stress introduced by submersible pumps on *Spirulina* sp. cultures already aerated by "air pumps" (small diaphragm compressors used in aquarium systems), comparing trichome length and overall productivity of the system.

2. MATERIAL & METHODS

Spirulina sp. LEB 52 strain was used for the kinetics tests and evaluation of trichome shear. 2L beakers were used as bioreactors, with a useful volume of 1.5l, in an open cultivation system with an initial cell concentration of 0.2 g L-1 (5) . The beakers, organized in triplicates, were aerated using submerged aquarium pumps (OT-1000F - 650 L h^{-1} – OceanTech©), and an external aeration pump (S-510 - 4 L min^{-1}- Boyu©). The controls had only the external aeration pump (S-510 - 4 L min^{-1}- Boyu©) connected to the culture with hoses. All the systems were kept under the same conditions of variable (ambient) temperature and lighting, in a greenhouse. Culture samples were done at the start of the experiment (t0) and then at 30 minutes, and 1; 2; 4; and 6 hours after the experiment was launched. Further samples were taken every day for 7 days to assess growth and trichome development.

Growth was assessed by gravimetry, using a 0.7 µm pore glass fiber membrane, by optical density (measured at 680 nm using a 96-well microplate reader), and by counting and measuring the trichomes. To this end, microscopy of these samples was carried out using a Neubauer chamber. The trichomes were measured and counted using ImageJ© software, with photos taken from the microscopy. Using the collected growth and shear data, growth curves were developed to compare the kinetics of the submerged pump and the external pump, as well as the variation in size and number of trichomes over time in both tests, allowing for indirect evaluation of the shear over the cyanobacteria. The graphs were created using OriginPro© software.

3. RESULTS & DISCUSSION

The trichome count using a Neubauer chamber was developed based on a methodology adapted from the non-filamentous cell count technique. For this purpose, photographs were taken of two alternating quadrants at the ends of the chamber. Using ImageJ© software, a 0.25 mm line (the smallest square side) was used for calibration. Linear measurements were then taken from one end of the trichomes to the other. The trichome size values for each quadrant were summed up and divided by the total number of trichomes, thus giving the average trichome size per sample. The number of trichomes was calculated as follows (6) :

$$Number\ of\ trichomes/ml = \frac{Total\ of\ trichomes\ x\ 10000}{Number\ of\ squares\ counted}$$

Differences in the size and number of trichomes were observed during microscopy. The beginning of the experiment showed fewer trichomes and similar average sizes (Figures 1 and 3). The control experiment, after 7 days of continuous aeration, showed a larger number of trichomes, with a slightly larger average size (Figure 2). The pump experiment, after 7 days of aeration *and* pump agitation, showed smaller trichomes in large quantities (Figure 4). This increase in the number of trichomes is consistent wit trichome breakage and may be related to the shear in the pump.

Figure 1 First day of the control sample

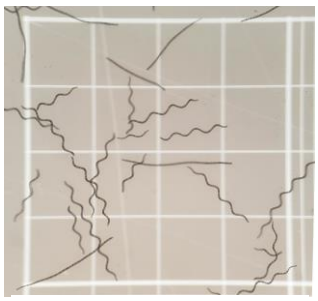

Figure 2 Last day of the control sample

Figure 3 First day of submerged pump sample

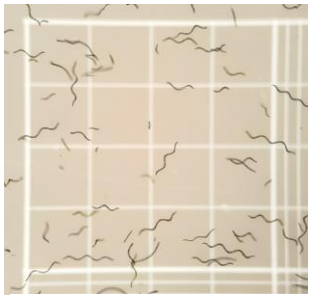

Figure 4 Last day of submerged pump sample

During the sampling period, between 11 a.m. and 12 p.m., the ambient temperature fluctuated between 15 and 35ºC, with an average of around 27ºC. However, during night and on colder days, the ambient temperature dropped to around 9ºC (Simepar). The internal temperature of the cultures varied between 13.6 and 26.6ºC for the control beakers (without a submerged pump) and between 22 and 32ºC in the beakers with a submerged pump, maintaining averages of 21.3ºC and 28ºC, respectively. The higher temperature condition in the beakers with the submerged pump may be due to the heating of the pump motor. The irradiation measured at noon varied between 460 and 1150 µmol photons m^{-2} s^{-1}, with a day length of 10.6h.

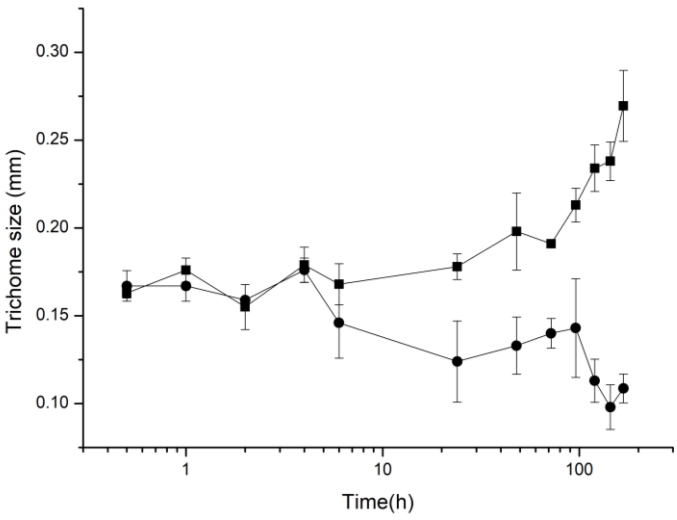

Figure 5 Trichome average size during the cultures, (time in logarithmic scale). ■ control (aerated), ● cultures with an added submerged pump.

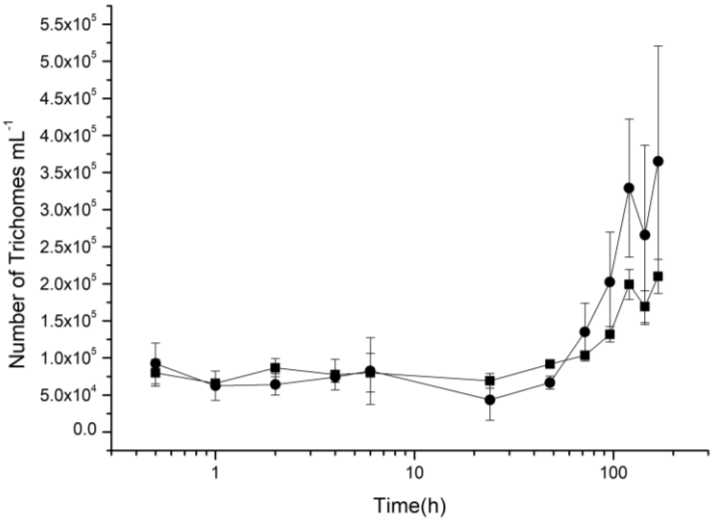

Figure 6 Cell concentration (as trichome number) throughout time (in logarithmic scale). ■ control (aerated), ● cultures with an added submerged pump.

The average initial number of trichomes was practically the same for samples without and with a submerged pump, $7.42x10^4 \pm 1.44x10^3$ trichomes mL^{-1} and $5.67x10^4 \pm 1.84x10^4$ trichomes mL^{-1}, respectively. On the last day, the number of trichomes was higher in all tests, reaching $2.10x10^5 \pm 2.29x10^4$ trichomes mL^{-1}, for the control beakers and $3.65x10^5 \pm 1.56x10^5$ trichomes mL^{-1}, for the samples with the submerged pump (figure 5). In the submerged pump tests, the average size of the trichomes *decreased* from 0.164 ± 0.01 mm initially to 0.100 ± 0.008 mm at the end of 7 days. In the control group, trichomes increased from 0.160 ± 0.003 mm at point initially to 0.269 ± 0.02 mm at the last point (figure 6).

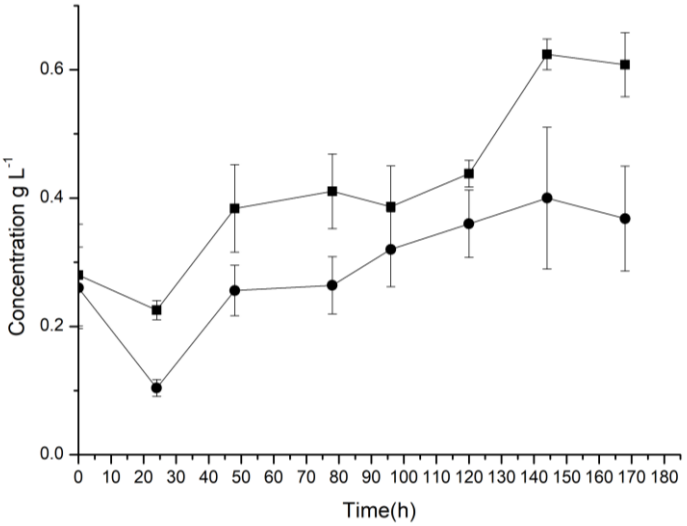

Figure 7 Biomass concentration during the experiment: ■ control (aerated), ● cultures with an added submerged pump.

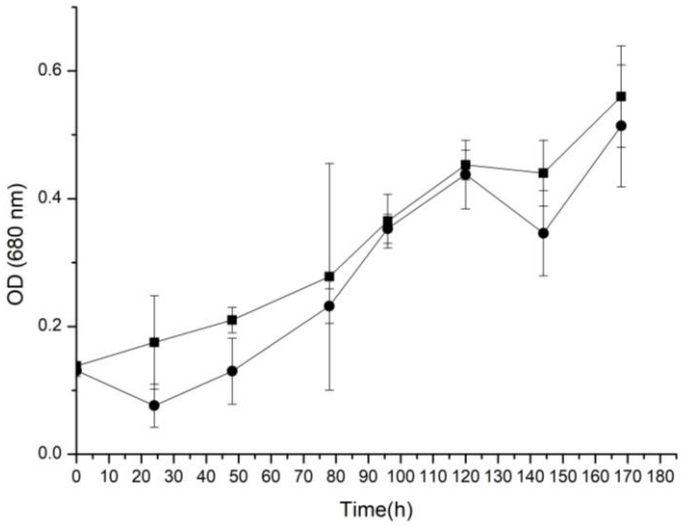

Figure 8 Optical density (OD) during the experiment. ■ control (aerated), ● cultures with an added submerged pump.

Although the submerged pump impacted the integrity of the trichomes, the biomass of *Spirulina* grown under these conditions increased. Initially, in both cultivation systems, the biomass decreased on the second day, likely due to an adaptation phase to the applied conditions, especially the lower temperatures. There was also a loss of water during the cultivation period, which was replenished and adjusted to maintain nominal volumes. A maximum concentration of 0.40 ± 0.08 g/L was reached in 6 days, starting from an initial concentration of 0.26 g/L in the submerged pump cultures. The control cultures showed greater growth, starting from an initial concentration of 0.28 g/L and reaching 0.62 ± 0.024 g/L, also in 6 days of cultivation (figure 7). The optical density readings were also proportional to growth, but errors can occur in this form of analysis due to the sedimentation capacity of the cyanobacteria cells, which affects the spectrophotometer readings (figure 8).

4. CONCLUSION

The use of submerged pumps can be beneficial in microalgae cultivation due to increased agitation, homogenization of the culture, and enhanced light absorption by all cells, especially in large-scale cultivations where the water column tends to be higher. However, using these pumps for filamentous cyanobacteria impacts cultivation due to the shear of the trichomes and decreased productivity. It should be noted that the high shear may have had a greater impact due to the small volume of the cultures in question; larger culture volumes might suffer less from this shear. Nonetheless, the negative effects were not sufficient to cause cell death and culture loss, which are important considerations when using submerged pumps in large-scale cultivation of *Spirulina* sp. Future experiments can focus on longer experiments to verify if adapted cultures could better withstand pump shear.

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