“Chlorella vulgaris (SAG 211-12) production under mixotrophic conditions for biofilm photobioreactor optimization”

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Introduction

Microalgae, especially Chlorella vulgaris strains, are important sources of valuable products and services. In recent years research has been carried out to develop biotechnologies capable of efficiently producing a large amount of microalgal biomass. In this context, biofilm cultivation and mixotrophy can reduce costs and increase production. A biofilm photobioreactor (RFPPB) was successfully developed using C. vulgaris strain SAG 211-12 [1] and performance can possibly be optimized under mixotrophic growth conditions. Nevertheless, this approach was not yet tested. Moreover, as mixotrophic cultures are prone to contamination, adequate protocols for contaminant control are needed.

In this work, steps towards increasing RFPPB performance are being done studying the growth capacity of C. vulgaris (SAG 211-12) in photoautotrophic and mixotrophic cultures. Results so far obtained in suspended cultures are presented, which will be followed by biofilm assays.

Aim

➢ Increase productivity and decrease microalgae production costs
➢ Characterize growth and production of C. vulgaris SAG 211-12 in mixotrophy.
➢ Compare photoautotrophic and mixotrophic growth of C. vulgaris SAG 211-12 in suspended cultures as biomass dry weight, cell density, organic matter content and medium pH.
➢ Develop a protocol for contaminant decrease/removal in microalgal cultivation.

Methodology

1. Elaboration of washing-centrifugation cycle protocol to remove fungi contamination.
2. Bold Basal Medium (BBM) [2] used for photoautotrophic growth and BBM with 1g/L of glucose for mixotrophic growth, in triplicate 100 mL to 500 mL Erlenmeyer flasks, with an initial cell number of 1x10^6 cells/mL. An oscillator at 40 rpm, a light intensity of approximately 2100 lux, with a nictemeral cycle of 14hr light/8hr dark, were used (Fig 1).
3. Culture Optical Density (OD) at 680 nm was measured with a Spectrophotometer and cell density was evaluated counting cells in a Hemocytometer. Medium pH was also measured.
4. Biomass dry weight (DW, mg/L) was calculated for each photoautotrophic and mixotrophic culture flasks.
5. Correlations among parameters were established.

Results

➢ Good correlation cell concentration - optical density in both culture modes:
  \[ Y = 3\times10^x + 373529 \quad R^2 = 0.9939 \quad \text{for Photoautotrophy} \]
  \[ Y = 8\times10^x - 2\times10^7 \quad R^2 = 0.9822 \quad \text{for Mixotrophy} \]
➢ Mixotrophic cultures reach exponential phase earlier and have a higher biomass production (3 times more) than photoautotrophic cultures. Maximum values attained were 595 mg/L and 211 mg/L, respectively (Fig 2 and Fig 3).
➢ pH differences were substantial throughout the experimental days. At the 10th day, the mean pH of photoautotrophic cultures was 7.29 and for mixotrophic was 8.97.
➢ Fungi and excess bacterial load were successfully controlled by the decontamination protocol developed for inoculum preparation for sub culturing.

Conclusion

This experimental work shows that mixotrophy enables a higher and faster growth of Chlorella vulgaris SAG 211-12 due to the addition of an organic carbon source such as glucose. This is an indicator that mixotrophy can have a crucial role to increase bioreactor performance in suspension cultivation mode. Research under mixotrophic biofilm conditions is ongoing. Inoculum decontamination protocol was successful.

References