Abstract

Optimization of Enzyme Production of *Trichoderma atroviride* Using Response Surface Methodology [†]

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Trichoderma atroviride is a well-known filamentous fungus used as biocontrol due to its capacity to produce various enzymes that degrade the cell wall of phytopathogenic fungi [1]. It is also an important microorganism for the industrial production of enzymes and metabolites. Light, especially blue light, is known to regulate the expression of many enzymes [2]. By means of response surface methodology (RSM), we aimed to optimize the production of enzymes of *T. atroviride*, using blue light and side streams from a flourishing industry. A three factors, two levels RSM was used for the enzyme production of *T. atroviride*, which provided a total of 25 experiments, generating relevant combinations among the 3 variable factors. *T. atroviride* was incubated for 10 days at 28 °C in minimal medium (MM) supplemented with 20% whey and 1% yeast extract. After 5 days, the samples were exposed to blue light, with variations in the light intensity and exposure time. To analyse the effect of irradiation on enzyme production over time, the irradiated samples were incubated for various durations, according to the factorial model. The protease activity was assayed using casein and Folin–Ciocalteu reagent against a tyrosine (Tyr/Y) standard curve [3]. The cellulase activity was assayed using carboxymethylcellulose (CMC) and DNS (3,5-dinitrosalicylic acid) reagent against a glucose standard curve [4]. Blue light was found to influence the protease and cellulase activity. The ANOVA analysis provided information about the influence of variables on the enzyme production of *T. atroviride*, along with their synergic effect. Both numerical and graphical results from data processing highlighted a relevant ratio between the variable factors which increased the enzyme production yield. By means of response surface methodology, we developed a biotechnological method for using byproducts resulting from the dairy industry, using blue light to induce enzyme production in *Trichoderma* cultures.

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