Effect of oxygen transfer rate on cellulases production in stirred tank and internal-loop airlift bioreactors

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In an aerobic process, such as enzymes production by fungi, the oxygen supply into fermentation medium is an important factor in order to achieve good productivities. Oxygen has an important role in metabolism and microorganism growth, being of extreme importance the control of both the dissolved oxygen transfer rate into the bioreactor and the oxygen consumption by the microorganism [1,2]. Dissolved oxygen transfer rate can be analyzed and described by means of the mass transfer coefficient, K_La, being one of the most important parameters for the design and operation of mixing/sparging of aerobic bioreactors.

In this study, two batch fermentations were performed using a stirred tank bioreactor (STB 8 L with a working volume of 5 L) and an internal loop airlift bioreactor (ALB 9.5 L with concentric draft tube, designed and constructed at the Department of Biological Engineering in the University of Minho (Pt) with a working volume of 6 L). Different K_La values were evaluated in attempts to optimize and compare the activities of extracellular cellulases synthesized by the fungus *Aspergillus niger* van Tieghem in STB and ALB.

The fermentations were performed at 30ºC using SR (Segato-Rizzatti) medium, at pH 6.0, containing 1% (w/v) of corncob as carbon source. On STB the K_La values used were: 12 h^{-1} (300 rpm; 0.2 vvm), 17 h^{-1} (300 rpm; 0.4 vvm), 25 h^{-1} (400 rpm; 0.2 vvm) and 30 h^{-1} (400 rpm; 0.4 vvm); and on ALB the K_La values used were: 5.0 h^{-1} (0.2 vvm), 6.5 h^{-1} (0.3 vvm), 9.0 h^{-1} (0.4 vvm) and 12 h^{-1} (0.5 vvm). Dissolved-oxygen and pH was monitored using Mettler-Toledo probes. One milliliter of antifoam 204 (Sigma) was used at the beginning of fermentation, which are performed during 15 days with samples collected each 24 h. Samples were filtered and used for enzymatic assays. Cellulase activity was determined as described by Miller [3] using Whatman® nº 1 filter paper, as substrate, at 55ºC for 60 minutes. β-glucosidase activity was determined as described in Kersters-Hilderson et al. [4] using 5 mM p-nitrophenyl-β-D-glucopyranoside as substrate, at 50ºC for 10 minutes. One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1 µmol of product per minute under the assay conditions and the activities were expressed in U L^{-1}.

Results showed that the highest cellulase and β-glucosidase levels were detected at the days 9 and 14-15 of fermentation, respectively; and the highest enzymatic levels were observed on ALB (1400 U L^{-1} cellulase and 6000 U L^{-1} β-glucosidase with a K_La of 5.0 and 6.5 h^{-1}, respectively). Although using these K_La where the dissolved oxygen transference was limited, the production of cellulase and β-glucosidase were 30% and 40% higher, respectively, in ALB than STB. These work shows that besides the dissolved oxygen transference other factors can affect the enzyme production, such as the type of bioreactor where the shear stress caused by the turbine on mycelia on STB could have a great influence.

Keywords: bioreactors; K_La; cellulase; *Aspergillus*; corncob.

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