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, $K_{La}$, which is 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 6 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 30°C using SR (Segato-Rizzatti) medium, at pH 6.0, containing 1% (w/v) of corn cob 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 55°C for 60 minutes. $\beta$-glucosidase activity was determined as described in Kersters-Hilderson et al. [4] using 5 mM p-nitrophenyl-$\beta$-D-glucopyranoside as substrate, at 50°C for 10 minutes. One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1 $\mu$mol of product per minute under the assay conditions and the activities were expressed in U L⁻¹.

Results showed that the highest cellulase and $\beta$-glucosidase levels were detected at the days 9 and 14-15 of fermentation, respectively; and the highest enzymatic levels were observed on ALB (400 U L⁻¹ cellulase and 6000 U L⁻¹ $\beta$-glucosidase with a $K_{La}$ of 3.0 and 6.5 h⁻¹, respectively). Although using these $K_{La}$ where the dissolved oxygen transference was limited, the production of cellulase and $\beta$-glucosidase were 30% and 40% higher, respectively, in ALB than STB. This 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; corn cob.

References

Effect of the combined treatment of ethanol and low pH in successive cycles on the growth of yeast and bacterial contaminants from the ethanol fermentation

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The alcohol-producing units in Brazil did not utilize sterile conditions during the fermentation, which results in contamination with undesirable yeasts and bacteria. For the latter, the acid treatment that is performed between the fermentative cycles with sulphuric acid (pH 2.0-2.5) inhibits their growth mostly, however, with the contaminant yeasts this procedure did not work. Dekkera yeasts have appeared as important contaminants of wine and fuel alcohol fermentations due to their ability to grow in fermentation limiting conditions with great efficiency. Previous study has indicated that a combined action of low pH and ethanol could inhibit the growth of Dekkera bruxellensis during the fermentation when applied to the yeast mass recovered after each fermentative cycle (Bassi et al. 2013). In the present work, the effect of a sulphuric acid solution in pH 2.0 + 13% ethanol over the yeast and bacterial growth were evaluated in five successive cycles of treatment in an experiment consisted of equal concentrations of Saccharomyces cerevisiae (strain CCA155 isolated from the alcoholic fermentation) and Lactobacillus fermentum (ATCC12925) in mixed culture. The microorganisms were previously cultivated separately in sugar cane juice medium (about 40 g/L of reducing sugars) at 30°C, 160 rpm until a concentration of 10⁸ cells/mL was reached. Equal volumes of the cell suspensions were resuspended in a sulphuric acid solution (pH 2.0) added with 13% ethanol v/v in a final volume of 50 mL in Erlenmeyer flasks and incubated at 30°C for 90 minutes at 160 rpm, in duplicate. Following the flasks were centrifuged, the cells were washed with distilled water twice and inoculated in sugar cane medium in the same conditions above described for 12 h to evaluate the recovery of cells after the treatment. The whole procedure was repeated five times. Samples were taken before the treatment (initial), after the treatment (final) and after the growth in sugar cane medium (recovery) and plated on YPD medium without and with actidione (for S. cerevisiae and D. bruxellensis, respectively) and MRS medium (for bacteria) to estimate the number of colony forming units. The acid-ethanol treatment inhibited completely the growth of L. fermentum in the first cycle and no cell recovery was observed until the end of the 5th cycle. The yeasts displayed a different pattern along the treatment cycles (Figure 1). For S. cerevisiae, a decrease in CFU was observed after each treatment cycle but the number of colonies increased progressively after each recovery step in sugar cane juice medium. After the 2nd cycle, the number of UFC was quite similar than the initial value. However for D. bruxellensis, at the end of the 5th cycle, the number of CFU was greatly lower than the initial value. The capacity of recovering from the stressful conditions in successive cycles of cell treatment is higher for S. cerevisiae than for D. bruxellensis. In summary, the combined effect of low pH and ethanol had significant effects on the yeast and bacterial contaminants without major effects over the main yeast. This procedure will be further applied to fermentation tests. Support: FAPESP (2011/17928-4; 2012/03401-3 and 2012/16258-4).

References

Figure 1. Yeast number during the successive cycles of acid-ethanol treatment.