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# Design of a novel Couette flow bioreactor to study the growth of fungal microorganism

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ABSTRACT

Cultivations using *Trichoderma reesei* Rut C-30 were performed in a 5-l Couette flow bioreactor (CFB) which was designed and built to perform experiments in batch and continuous modes. Process parameters such as dissolved oxygen, pH and temperature were measured and controlled without disturbing the shear profile inside the bioreactor. Effect of shear on the growth, protein production and morphology was studied by performing runs at 100, 200, 300 and 400 rpm. At higher shear rates, lower protein production rate and activity, and higher rate of fragmentation were observed. Also, the cell thickness decreased with increasing speed, going from 8.3 μm for the experiment at 100 rpm to 4.3 μm at 400 rpm. The effect of substrate, lactose (an inducer) or glucose, was investigated by switching the feed medium during the two runs performed at 300 and 400 rpm. The novel design of the CFB used in the present study includes a large volume that allows growing larger size microorganisms (e.g. fungi) and permits larger sampling volumes without affecting the cultivation. It also has the ability to carry out experiments for long periods of time, both in batch and continuous modes.



## 1. Introduction

Achieving adequate mixing is very important in submerged cultivation. Higher mixing intensity will certainly improve mass transfer. However, higher mixing intensity may adversely affect the growth of microorganism and product formation in addition to lead to higher energy requirements. To find a judicious intensity of mixing for a particular cultivation and type of mixing device, it is thus important to study the effect of shear on the growth, productivity and morphology of microorganisms. However, most of the studies reported in the literature were performed in conventional bioreactors, such as stirred tank bioreactor with Rushton turbines, where microorganisms are subjected to non-uniform shear fields (Reuss et al., 2000). Also, gas sparging required for oxygen supply may cause shear damage to the microorganism (Cherry and Hulle, 1992) due to the shear forces associated with bubbles rising through the cultivation medium and bubbles bursting at the liquid surface. Further, it is difficult to evaluate the effect of shear due to mixing and gas sparging in these types of bioreactors. In order to overcome these limitations, several studies have been performed in a Couette flow bioreactor (CFB) (Janes et al., 1987; O'Connor et al., 2002; Sahoo et al., 2003; Sun and Linden, 1999). In a CFB, the cultivation broth is entrapped in the small annular section of the two concentric cylinders with inner or outer cylinder rotating at a constant speed. For a thin annular space, almost constant shear rate can be assumed (Bird et al., 2006). Indeed, operating coaxial cylinder bioreactors with the outer cylinder rotating leads to laminar Couette flow and thus uniform shear stresses within the annulus (Curran and Black, 2005). A bioreactor with an outer rotating cylinder provides operations at higher Reynolds numbers (Schlichting, 1955) compared to bioreactors with an inner rotating cylinder. In this study, aeration is provided by diffusing air through a membrane fixed to the external surface of the inner cylinder. Oxygen flux to the cultivation broth can be varied by manipulating the pressure or by changing the oxygen content of the feed gas.

CFBs have been successfully used in the past to study the effect of shear on the growth of different microorganisms (Sahoo et al., 2003; Wong et al., 2001). However, these bioreactors were small in size, typically less than 1 l, and usually run in batch mode. Another constraint in the study of shear effect on microorganisms lies in the fact that its effect is time-dependent (O'Connor et al., 2002) and, therefore, it is important to perform these studies by exposing the cells to similar residence times as would prevail during the cultivation. Considering all of the above-mentioned characteristics and constraints, the novelty in the CFB designed for this investigation consists in its larger volume (5 l) compared to previous studies and that it can be operated in batch or continuous mode for days. Process parameters such as pH, dissolved oxygen and temperature are

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**Nomenclature**

| | |
|---|---|
| $A$ | surface area available for aeration ($m^2$) |
| $C_L$ | dissolved oxygen concentration (mol $m^{-3}$) |
| $C_L^*$ | dissolved oxygen concentration in equilibrium with mean gaseous oxygen concentration (mol $m^{-3}$) |
| CFB | Couette flow bioreactor |
| DO | dissolved oxygen (%) |
| $k$ | ratio of inner to outer radii of the concentric cylinders |
| $K_L$ | oxygen mass transfer coefficient (m $s^{-1}$) |
| $K_{L,e}$ | effective oxygen mass transfer coefficient (m $s^{-1}$) |
| $K_La$ | overall oxygen mass transfer coefficient ($s^{-1}$) |
| $K_{L,e}a$ | overall effective oxygen mass transfer coefficient ($s^{-1}$) |
| $N_{O_2}$ | oxygen flux density (mol $m^{-2}$ $s^{-1}$) |
| OUR | oxygen uptake rate (mol $l^{-1}$ $s^{-1}$) |
| $P_M$ | membrane permeability (m $s^{-1}$) |
| $r$ | radial distance in the annular section between two concentric cylinders (m) |
| $r_o$ | radius of outer cylinder (m) |
| $r_i$ | radius of inner cylinder (m) |
| Re | Reynolds number |
| rpm | revolution per minute |
| Sc | Schmidt number |
| Sh | Sherwood number |
| $t$ | time (s) |
| $V_L$ | liquid volume in the bioreactor ($m^3$) |

*Greek letters*

| | |
|---|---|
| $\dot{\gamma}$ | shear rate ($s^{-1}$) |
| $\Omega$ | angular velocity (rad $s^{-1}$) |

also measured and controlled throughout the cultivation without disturbing the shear field.

In the present study, the application of CFB for the study of shear stress on filamentous microorganism was done by performing experiments using the industrially important fungi *Trichoderma reesei* used for the production of cellulase enzyme. Mitard and Riba (1988) performed experiments in a CFB using *Aspergillus niger* but oxygen was provided by sparging air and the morphology analysis reported in their study was performed on the pellet form instead of filamentous form encountered in *T. reesei* cultivation. Thorough investigations have been performed involving biomass and protein production but very few studies involve correlating morphology, growth and enzyme production. In the present study, samples were analyzed for biomass, protein production and activity, and the influence of shear on the morphology of microorganism was also examined.

## 2. Materials and methods

The strain used was *T. reesei* Rut C-30 (ATCC 56765). The culture medium (5 l) contained: lactose, 5 g $l^{-1}$; $(NH_4)_2SO_4$, 1.4 g $l^{-1}$; $KH_2PO_4$, 2.0 g $l^{-1}$; $MgSO_4{\cdot}7H_2O$, 0.3 g $l^{-1}$; $CaCl_2{\cdot}2H_2O$ (autoclaved separately), 0.3 g $l^{-1}$; $FeSO_4{\cdot}7H_2O$, 5.0 mg $l^{-1}$; $MnSO_4{\cdot}7H_2O$, 1.6 mg $l^{-1}$; $ZnSO_4{\cdot}7H_2O$, 1.4 mg $l^{-1}$; $CoCl_2{\cdot}6H_2O$, 2 mg $l^{-1}$, peptone, 2 g $l^{-1}$ and yeast extract, 0.5 g $l^{-1}$. The pH of the medium was initially adjusted to 4.0. Medium was autoclaved for 20 min and then transferred to the bioreactor under sterile conditions. Shake flask cultures were performed with a volume of 250 ml in a 1-l Erlenmeyer flask with three baffles. Flasks were kept in an orbital shaker at 200 rpm and 28 °C for 55 h.

### 2.1. Sample analysis

A 25-ml sample was collected approximately every 12 h. One ml of each sample was used immediately for image analysis and the remaining sample was stored at 4 °C pending further analysis. The amount of biomass was quantified by dry weight analysis. Protein concentration was determined using the Bradford assay with bovine serum albumin (BSA) as standard. Filter paper (FPA) and carboxymethylcellulose (CMC) activities were determined as per the IUPAC protocol (Ghose, 1987). Glucose and lactose concentrations were determined using YSI analyzer (YSI Incorporated, USA) and enzymatic kit (Boehringer Mannheim, Germany), respectively. Image analysis was performed according to protocol described by Lecault et al. (2007).

### 2.2. Design of the Couette flow bioreactor

Shear rate at any radial position '*r*' in the annular section between two concentric cylinders with a rotating outer wall can be calculated by Eq. (1).

$$\dot{\gamma}(r) = 2\Omega\frac{(kr_o/r)^2}{1-k^2} \quad (1)$$

where $k$ is the ratio of inner to outer radii ($r_o$) and $\Omega$ is the angular velocity of the outer cylinder. For a small annular space, $k$ tends to one and as shown in Eq. (1), the shear rate is nearly constant (Sahoo et al., 2003). An inner radius of 8.89 cm (3.5″) and an outer radius of 10.16 cm (4″) were selected to give a value of $k$ equal to 0.875. The resulting average shear rates at 100, 200, 300 and 400 rpm are 78.1, 156.3, 234.5 and 312.6 $s^{-1}$, respectively. These shear rate values for the CFB designed in the present study correspond to average shear rates typically encountered in a stirred tank bioreactor (Metzner and Taylor, 1960). The effective working height of the reactor was approximately 66 cm to provide a working reactor volume of 5 l. In this work, shear rate has been calculated on the assumption that the medium is Newtonian, which is acceptable (based on the previous studies performed in our laboratory (Malouf, 2008)) due to the relatively low biomass concentration (maximum 3.0 g $l^{-1}$) of *T. reesei* obtained in the CFB. Similar observations and assumptions have been made at low biomass concentration for other filamentous microorganisms (Jüsten et al., 1996).

There are two main design considerations regarding oxygen supply. First, the oxygen supply should be able to meet the oxygen demand of the microorganisms and, second, the oxygen needs to be supplied by diffusion through the membrane rather than bubbling. In a hydrophobic microporous membrane, the pores are filled with gas and water does not wet its surface. This is preferred in membrane bioreactors since it decreases the attachment of microorganism on the membrane surface (bio-fouling) and also, the mass transfer resistance is considerably smaller than in a hydrophilic membrane, where the pores are filled with water (Côté et al., 1988; Schneider et al., 1995). However, one of the disadvantages of these membranes is that the pressure on the gas phase cannot be increased above the bubble point because gas bubbles may appear in the liquid (low bubble point). On the other hand, dense hydrophobic membrane allows operation at higher gas pressure to achieve adequate aeration without forming air bubbles. However, the gas film resistance of microporous membrane is 10–150 times smaller than the resistance of dense membranes (Reij et al., 1998). To take advantage of both types of membranes, a composite membrane consisting of a thin top layer (<1–30 μm) of dense material over a highly porous support layer can also be used. Though Schwarz and Anderson (1998) have suggested that if the pore size is 1 μm or less, a coating of dense material is not preferred. In the present study, a commercially available microporous hydrophobic PTFE membrane with 0.2 μm pore size and 139 μm