Invited paper

Slurry Bioreactor Design for Shear-Sensitive Mycoprotein Production

Y. Chisti and M. Moo-Young

Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario,
N2L 3G1, CANADA

The effects of impeller associated agitation on fermentations of shear-sensitive mycelial microfungus Neurospora sitophila *are demonstrated. The impact of agitator type and the agitation rate on biomass protein production and cellulose utilization are shown in a relatively large 75 l fermenter. Results from cellulosic solid-substrate fermentations are presented. These fermentations tend to be unusual in that the broths contain solid-substrate particulates in addition to fungal mycelia and this leads to more complex rheological (and hence shear related) behaviour than in typical fungal cultures.*

All microorganisms, animal and plant cells are sensitive to various levels of hydrodynamic and mechanical forces. In fact, disruption of cells by fluid-shear, solid-shear or impact are established operations in bioprocessing [1]; however, in production of microbial or eucaryotic cells and their metabolites, exposure of the biocatalysts to high shear fields can be counterproductive [2,3]. Investigations of the safe limits of exposure of biocatalysts to fluid mechanical forces and design of bioreactors to not exceed the identified limits is of increasing importance especially because the newer genetically modified biocatalysts tend to be less robust than their wild counterparts [4,5]. Different morphological forms of the same organism may show different susceptibility to mechanical damage [6].

This paper demonstrates the effects of impeller associated agitation on slurry fermentations of the mycelial microfungus Neurospora sitophila. The effects of agitator type and the agitation rate are shown in a relatively large 75L fermenter. The food-grade fungus N. sitophila is of potential commercial significance in the context of a process developed for the conversion of lignocellulosic agricultural materials to protein-rich foods and feeds [2].

MATERIALS AND METHODS

Cultures and inocula

The microfungus Neurospora sitophila (ATCC 36935) was maintained at 4°C in submerged culture on glucose (10 kgm^{-3}) supplemented with yeast extract (Diffco) (2 kgm^{-3}) and the following nutrient salts (per litre): $(NH_4)_2SO_4$, 0.47 g; urea, 0.86 g; KH_2PO_4, 0.714 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.2 g; $FeCl_3$, 3.2 mg; $ZnSO_4 \cdot 7H_2O$, 4.4 mg; H_3BO_3, 0.114 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.48 mg; $CuSO_4 \cdot 5H_2O$, 0.78 mg; $MnCl_2 \cdot 4H_2O$, 0.144 mg. Inocula were grown at 26°C on the specified carbon source (5 kgm^{-3}) supplemented with 0.5 kgm^{-3} molasses (Hoffman Feeds Ltd, Heidelberg, Ontario) and the earlier specified salts.

The fermentation media contained a carbon source ("Solka Floc" wood cellulose or molasses). The Solka Floc cellulose (α-cellulose) was made from

E. Galindo and O.T. Ramírez (eds.), Advances in Bioprocess Engineering. 25-28.

wood pulp (James River Corporation, Berlin, New Hampshire). The KS1016 grade used in this work had average particle (fibre) length of 290 μm and a degree of crystallinity of 75-77% crystalline cellulose. Although the media were supplemented with the earlier specified nutrient salts, the entire complement of salts was not necessary for the naturally occurring substrates (*e.g.*, corn stover); only ammonium sulfate and phosphates were essential.

Fermentation conditions

Fermentations were conducted either in shake flasks or in a 75 L (nominal) stirred tank fermenter (MBR Sulzer, Switzerland). The shake flask runs were performed in 250 mL flasks containing 100 mL medium including an specified carbon source and the nutrient salts. The flasks were sterilized at 121°C for 30 minutes, cooled to ambient, inoculated and held at the specified temperature on a gyratory shaker at 250 rpm. Unless otherwise indicated, the pH at inoculation was 6.0. At desired times, the flasks were rapidly cooled and stored at 4°C if necessary. The flasks were analyzed for total dry solids, crude protein and cellulose.

Crude protein and cellulose

For crude protein and cellulose determinations, the fermentation broth was filtered under suction through a 25 μm "Nitex" nylon cloth (Thomson Co., Scarbrough, Ontario), the filter cake was washed with several broth volumes of deionized water and dried overnight at 90°C. The dry biomass was ground to 1 mm particle size and a portion was analyzed for total nitrogen using a microKjeldahl technique [7]. The crude protein content of the biomass were calculated as 6.25 × total nitrogen, and percent (w/w) protein as gram protein per 100 g total dry solids. The cellulose content were determined by the spectrophotometric anthrone-sulfuric acid method [8]; percent cellulose was calculated on the same basis as crude protein.

Shear effects

The influence of shear on protein production was investigated in the 75 L fermenter (vessel diameter = 0.318 m) with a final working volume (after inoculation) of 50 L fermentation broth. The temperature and pH were controlled at 26°C and pH 6.0, respectively. The dissolved oxygen level was not allowed to drop below 20% of air saturation. Aeration rate varied (0.4 - 0.8 vvm) in response to the dissolved oxygen level. A 6-blade disc turbine was used for agitation ($\underline{d}_i/\underline{D} = 0.57$; $\underline{C}_i = 0.6 \bullet \underline{d}_i$; $\underline{h}_L/\underline{D} = 1.9$) at 250, 300 or 350 rpm corresponding respectively to tip speeds of 2.35, 2.82 and 3.29 ms^{-1}. *N. sitophila* was grown on KS1016 grade Solka Floc (5 kgm^{-3}) supplemented with molasses (0.5 kgm^{-3}), $(NH_4)_2SO_4$ (0.28 gL^{-1}); urea (0.52 gL^{-1}), KH_2PO_4 (1.0 gL^{-1}) and other, previously listed, nutrient salts at half the concentrations specified earlier.

RESULTS AND DISCUSSION

Agitation conditions

Mechanical agitation in stirred fermenters is known to damage mycelial biomass and affect the yield of the product [3,5,9]. Characterization of the influence of the impeller speed on *N. sitophila* protein production was required to identify the suitable operational conditions, any scale-up limitations and the sensitivity of this particular fermentation to impeller induced shear.

The protein production profiles at various agitation rates (tip speeds) are shown in Figure 1. For otherwise identical conditions, increasing tip speed of the Rushton disc turbine impeller lowered the rate of protein production (Figure 1), and the maximum protein yield. Thus, as shown in Table 1, the maximum specific protein production rate (μ) decreased from a high of 0.09 h^{-1} at 250 rpm to a low of 0.05 h^{-1} at 350 rpm. In relative terms, the protein production rate (μ_R) at the highest rpm was only 55% of that at the lowest agitation. Data on peak protein production and cellulose utilization (at 38 hours into the fermentation) are shown in Table 1 in absolute and relative terms. At the highest tip speed used (3.29 ms^{-1}) a distinct lag phase was noticed (Figure 1) in protein production compared to the results at lower agitation intensities. Clearly, the *N. sitophila* fermentations were quite sensitive to excessive agitation, and low agitation rates, consistent with adequate mixing and oxygen supply were indicated for the successful production process.

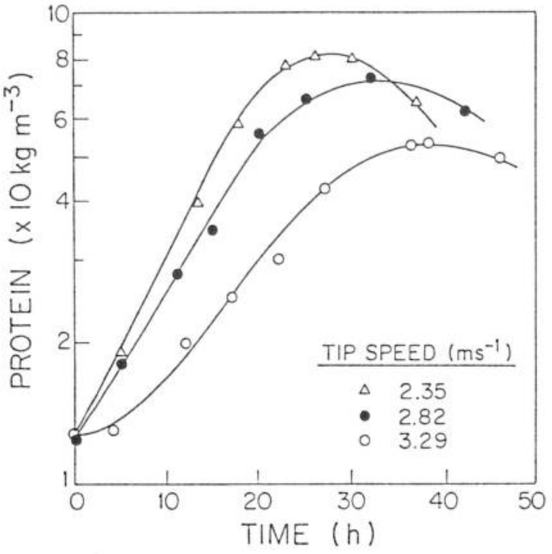

Figure 1. Effect of agitation on protein production. Impeller speed (rpm): (△) 250; (•) 300; and (○) 350. *N. sitophila* grown on KS1016 grade of Solka Floc wood cellulose (26°C, pH 6.0).

Table 1. Effect of agitation on protein production and cellulose utilization.

N rpm	μ h^{-1}	μ_R (-)	Crude Protein (%)	Cellulose Used (%)
250	0.09	21.0	31.1 (1)*	79.8 (1)*
300	0.07	0.78	27.7 (0.88)	69.0 (0.86)
350	0.05	0.55	21.2 (0.67)	55.6 (0.70)

* Values in parentheses are relative to the value at 250 rpm.

Identical fermentations conducted with the turbine replaced with an axial flow Prochem impeller (Figure 2) operated at 250 rpm gave marginally higher specific growth rate ($\underline{\mu} = 0.11$ h^{-1}) than was obtained with the Rushton turbines; however, the cellulose utilization was higher at *ca.* 86% with the Prochem impeller. Unlike the Rushton turbines, the axial flow impellers could be operated up to 400 rpm without any noticeable deleterious effects on the fermentations.

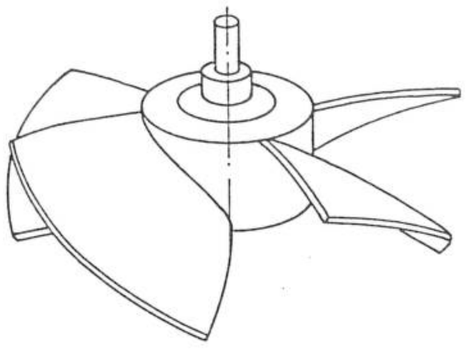

Figure 2. The Prochem Maxflo axial flow impeller.

CONCLUSIONS

Solid-substrate slurry fermentation systems for highly aerobic and shear-sensitive cultures of the mycelial fungus *Neurospora sitophila* present an unusually complex design problem. The fungal broths are highly viscous and non-newtonian because of the filamentous fungal solids and the cellulosic substrate fibres. Yet, the oxygen demand is high *and* the fungus is sensitive to agitation levels needed to provide sufficient oxygen transfer. Conventional, Rushton turbine agitated fermenters perform poorly for this system while simple airlifts have in the past been shown to be not particularly effective [10]. Redesign of stirred bioreactors with replacement of Rushton turbines with suitable axial flow devices improves bulk mixing in this rheologically complex fermentation. At the same time, the agitation-associated mechanical damage to mycelia is reduced. An alternative bioreactor configuration discussed elsewhere [10] and consisting of a concentric draft-tube airlift reactor with axial flow impellers located within the tube has also been shown to be effective for this fermentation at scales up to 1300L.

NOMENCLATURE

C_i	Impeller clearance above bottom of tank (m)
D	Diameter of fermenter (m)
d_i	Diameter of impeller (m)
h_L	Static liquid or slurry height (m)
N	Impeller speed (rpm)
μ	Specific protein production rate (h^{-1})
μ_R	Relative protein production rate (-)

LITERATURE CITED

1. Chisti, Y. and Moo-Young, M., Enzyme Microb. Technol., **8**, 194-204 (1986). Disruption of microbial cells for intracellular products.

2. Moo-Young, M., Chisti, Y. and Vlach, D., Biotechnol. Lett., **14**, 863-868 (1992). Fermentative conversion of cellulosic substrates to microbial protein by Neurospora sitophila.

3. Ujcova, E., Fencl, Z., Musilkova, M. and Seichert, L., Biotechnol. Bioeng., **22**, 237-241 (1980). Dependence of release of nucleotides from fungi on fermenter turbine speed.

4. Dunnill, P., Chem. Eng. Res. Des., **65**, 211-217 (1987). Biochemical engineering and biotechnology.

5. Moo-Young, M. and Chisti, Y., Biotechnology, **6**(11), 1291-1296 (1988). Considerations for designing bioreactors for shear-sensitive culture.

6. Morimura, S., Kida, K. and Sonoda, Y., J. Ferment. Bioeng., **74**, 129-131 (1992). The influence of shear stress and its reduction in the production of saccharifying enzyme from shochu distillery wastewater by Aspergillus awamori var. kawachi.

7. Lang, C. A., Anal. Chem., **30**, 1692-4 (1958). Simple microdetermination of Kjeldahl nitrogen in biological materials.

8. Updegraff, D. M., Anal. Chem., **32**, 420-4 (1969). Semimicro determination of cellulose in biological materials.

9. Chisti, Y. and Moo-Young, M., Biotechnol. Bioeng., **34**, 1391-1392 (1989). On the calculation of shear rate and apparent viscosity in airlift and bubble column bioreactors.

10. Moo-Young, M., Chisti, Y. and Vlach, D., Biotechnol. Adv., **11**, 469-479 (1993). Fermentation of cellulosic materials to mycoprotein foods.