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Submerged citric acid fermentation: rheological properties of *Aspergillus niger* broth in a stirred tank reactor

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Summary. A large number of submerged citric acid fermentations in a beet molasses substrate was studied. The development of *Aspergillus niger* from conidia to pellets was followed. Rheological characteristics of the fermentation broth including the pellets were determined. The results obtained confirm the fact that the non-Newtonian pseudoplastic behaviour of the fermentation broth was due to the presence of mycelial pellets. The most significant changes in rheological properties occurred during the period of maximal citric acid production and increase in biomass.

Introduction

The rheological behaviour of fermentation broth is of considerable importance in describing the transport phenomena in bioreactors. It relates to problems of mass transfer and heat transport as well as energy consumption. A fermentation broth is actually a suspension of microorganisms and, as observed by several authors (Karow et al. 1953; Deindorfer et al. 1955; Deindorfer and West 1960; Solomons and Weston 1961), it can exhibit, even with a low concentration of solids, marked non-Newtonian characteristics. In batch fermentation the rheological behaviour changes during fermentation because of changes in the microorganism concentration and morphological characteristics.

The physical properties of mycelial suspensions are rather different from bacterial or yeast cultures. In mycelial microorganisms two forms of growth can be distinguished: the filamentous and the pellet form. In pellet form the mycelium develops stable spherical aggregates consisting of a more or less dense, branched and partially intertwined network of hyphae (Berovič and Cimerman 1982). Such pellets can have diameters of up to several millimetres. A suspension of pellets is usually less viscous than suspensions of filamentous growth (Berovič 1986). For submerged citric acid fermentation the pellet growth form is widely recommended (Martin and Waters 1952; Clark and Lenz 1963; Metz et al. 1979; Berovič and Cimerman 1979, 1982).

The high viscosity and the non-Newtonian character of mycelial suspensions lead to difficulty in mixing, which in turn strongly affects interphase oxygen transfer. A higher viscosity demands a longer mixing time and also causes so-called "dead corners" in the bioreactor where the suspension can be stagnant for a considerable period of time. Stagnant zones in the bioreactor can easily decrease the productivity of the microorganism and cause the production of undesirable metabolites (Elmayergi and Moo-Young 1973).

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Materials and methods

All experiments were performed in a 7-l laboratory stirred tank reactor (STR; Bioengineering, Switzerland). *Aspergillus niger* strain A60 (NRRL 2270 Peoria), was used throughout all experiments. The initial spore concentration of the inoculum was 5×10^7 conidia/ml. The fermentation broth consisted of diluted beet molasses (12.5% reducing sugar). The optimal addition of $K_4[Fe(CN)_6]$ was determined for every molasses sample separately in shaken culture experiments. It was added in two stages (Berovič and Cimerman 1982). The fermentations were carried out at $T = 30°$ C, aeration rate (Qg) = 1 vvm and agitation (N) = 600 rpm. The growth of mycelia was followed by microscopic observations. Rheological measurements were performed using a viscometer Rheotest 3 in a double cylinder configuration (2 VEB MLW, Prüfgeräte-Werk, Medingen, FRG). A rotating cylinder S2 (d = 37.7 mm) and a measuring cell (D = 40.39 mm) were used.

Citric acid was determined as total acidity titrimetrically with 0.1 M NaOH. The reducing sugar content was determined by the standard Fehlings method and the biomass after drying at 105° C.

For the oxygen partial pressure measurements in the fermentation broth a polarographic electrode (Industrial Lab MFG 509 with IL amplifier Type 531; USA) was used.

The volumetric oxygen transport coefficient ($k_L a$) in the fermentation broth and the specific respiration rate ($Q_{O_2} X$) were determined by the dynamic method (Heineken 1971; Bandyopadyay and Humphrey 1967).

The shear stress (τ) of the fermentation broth was characterized by the power law model of Ostwald-de Waele (Blanch and Bhavaraju 1976).

$$\tau = K \cdot \dot{\gamma}^{n} \quad (1)$$

$\dot{\gamma}$ = shear rate.

The apparent viscosity (η_a) is expressed by Eq. 2:

$$\eta_a = K\dot{\gamma}^{n-1} \quad (2)$$

The rheological characteristics of the fluid consistency index (K) and the flow behaviour index (n) were evaluated from a log-log plot of Eq. 2.

Results and discussion

The germination of *A. niger* conidia was observed 6 h after inoculation. Swelling of the young hyphal agglomerates could be detected microscopically during the first 29 h of cultivation. This was the period of initial mycelial growth. After 36 h of cultivation, the microorganism was present in the form of spherical mycelial pellets that had developed from the swollen hyphal agglomerates, characterized by short, forked, bulbous hyphae orientated in all directions with areas of swollen cells showing more granulations and vacuoles. During the subsequent development of *A. niger* only the diameter of the pellets slightly increased. Pellets with short and thick peripheral hyphae were the characteristic productive mycelial forms in the STR.

The rheology of citric acid fermentation broth is strongly dependent on the development of the microorganism. During the first period of fermentation, 36 h after inoculation, the development from spores to hyphae was characterized by low biomass content up to 3 g/l (Fig. 1) with nearly Newtonian behaviour of the fermentation broth, showing a high flow behaviour index (n = 1.00–0.95) and a low fluid consistency index K = 5–10 mPas^n (Figs. 2, 3). During this period a relatively small difference in the k_La value of 20 h^{-1} was detected. A significant reduction in the oxygen partial pressure corresponded to a high $Q_{O_2}X$, which reached its maximum ($Q_{O_2}X = 0.052$ mg O_2 l^{-1} s^{-1}) during this period (Fig. 4).

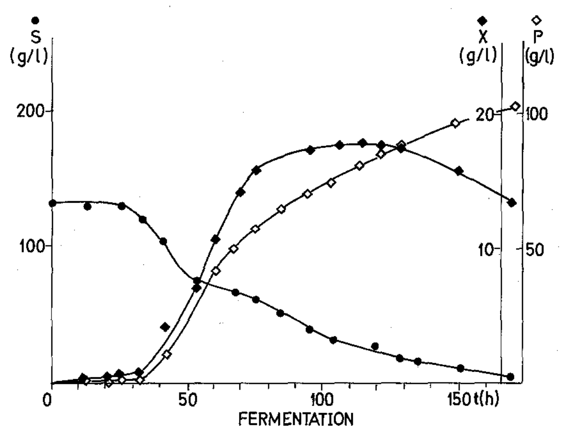

Fig. 1. The courses of substrate sugar concentration (S), biomass concentration (X) and citric acid formation (P) during the fermentation process in a stirred-tank reactor

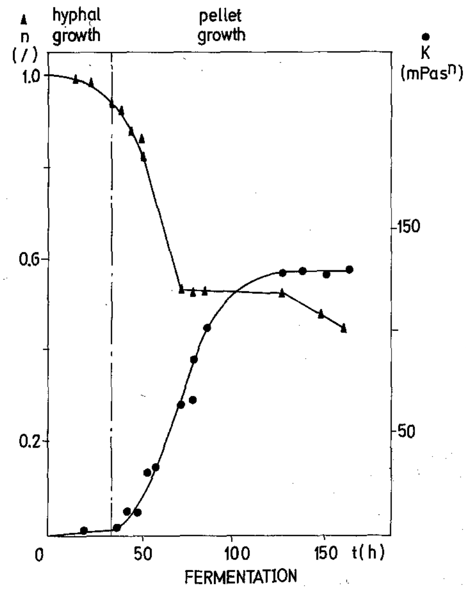

Fig. 2. The courses of the flow behaviour index (n) and fluid consistency index (K) of the fermentation broth during the whole fermentation period (shear rate, $\dot{\gamma}$ = 100–1000 s^{-1})

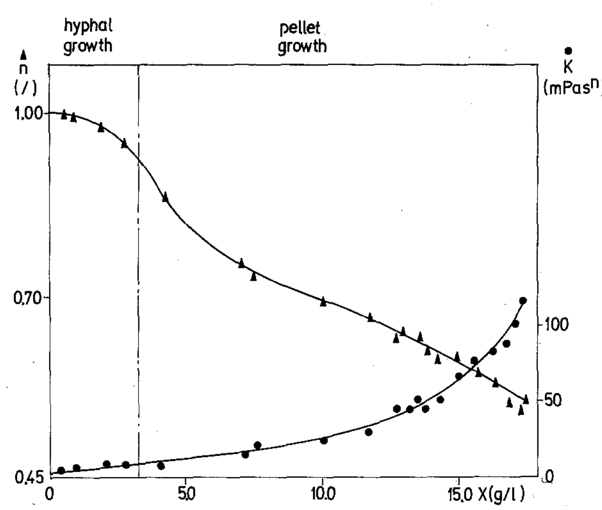

Fig. 3. Relationship between the flow behaviour index (n) and biomass concentration (X)

During the second fermentation period the change in *A. niger* morphology to spherical pellets and the increase in biomass from 3 to 17.5 g/l changed the rheological behaviour of the fermentation broth to a pseudoplastic one, reducing n from 0.95 to 0.45 with an increase in K to 120 mPas^n (Figs. 2, 3). In the second period intensive accumulation of citric acid also occurred (Fig. 1).

The changes in the rheological behaviour of the fermentation broth caused a significant reduction in the k_La from 156 to 28 h^{-1} and of the oxygen partial pressure to 6%. The changes in redox potential and pH related to the development of the microorganism during both stages of the fermentation.

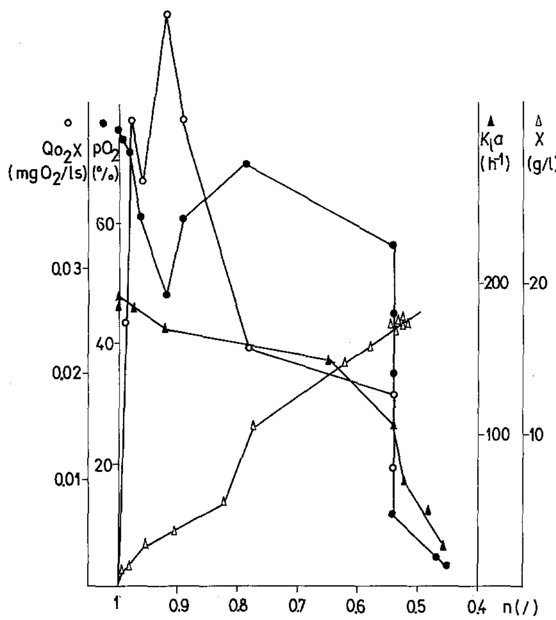

Fig. 4. Oxygen partial pressure (pO_2), volumetric oxygen transfer coefficient (k_La), biomass concentration (X) and specific respiration rate ($Q_{O_2}X$), related to the flow behaviour index (n)

In conclusion, the results presented confirm the fact that a submerged citric acid fermentation broth shows non-Newtonian rheological behaviour. This is demonstrated especially well during the period of change in mycelial growth from hyphal agglomerates to spherical mycelial pellets, which represent the productive form of the fungus. The most significant changes in rheological behaviour occurred during the period of maximal citric acid production at the time of exponential increase in biomass. It seems that the pellet growth form represents resistance to shear stress in the system, causing pseudoplastic rheology and significantly reducing the volumetric oxygen transfer and oxygen partial pressure.

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