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# STUDIES ON THE RHEOLOGICAL PROPERTIES OF THE FERMENTATION BROTH IN THE PRODUCTION OF PECTOLYTIC ENZYMES

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A b s t r a c t. The paper presents evolution of viscosity in a process of obtaining pectolytic enzymes in a submerged cultivation system with a storm of *Aspergillus niger*. Two groups of culture media were used: one with dry fermented apple pulp and wheatbran and the other with different saccharides as carbon sources. For the first group of media, the initial viscosity, expressed only in relation to shear rate was $10^4$-$10^5$ higher than the viscosity of the second group of media. In both cases, the time-increasing of the viscosity accompanies the microorganism groth.

K e y w o r d s: fungi, viscosity, shear rate

## INTRODUCTION

The microbial pectolytic enzyme were one of the first industrially produced and used in food and some fields of light industry [1,2,9 ].

Fungi were generally used to obtain these enzymes but specific features of mycelium groth determined special rheological properties of the culture broth, that is difficult to characterize. Studies on the rheological properties of the fungi culture broth are such as [3-6].

High broth viscosity determines difficulties in mass, oxygen and heat transfer, broth homogenity disturbing and shear stress induced by agitation [3,5,8 ].

Relation between shear stress and viscosity is specific for any non-newtonian fluid, therefore this relation needs to be experimentally determined for a particular type of fermentation [7,8 ].

The paper presents time-dependence of *Aspergillus niger* broth viscosity on the medium composition in the bioprocess of obtaining pectolytic enzymes.

## MATERIALS AND METHODS

Microorganism: a strain of *Aspergillus niger* isolated from the surface of depreciated carrots.

Culture media (w/v): $NH_4/_2SO_4$ 0.5%, $KH_2PO_4$ 0.5%, $CaCO_3$ 0.04%, NaCl 0.4%, $MgSO_4$ 0.03%; Carbon sources: their nature and concentrations (10-18%) are presented in Table 1.

**T a b l e 1.** Carbon sources and media variants

| Nature of carbon source | Dry fermented apple pulp + wheat bran | | | | Glucose syrup | Sucrose | Solid glucose | Mono + Oligo-saccharides |
|---|---|---|---|---|---|---|---|---|
| | | | | | Variant | | | |
| | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ | $V_7$ | $V_8$ |
| Content in carbon source (%) | 18.9 | 14.4 | 15.8 | 13.9 | 5.8 | 10 | 10 | 10 |

Final pH: 5.0-5,2 adjusted with $H_2SO_4$.

The experiments were conducted in a feed -batch bioreactor, working volume 62 l, at 30 °C, 12 rpm, 0.01 v/v/min.

The inoculum was prepared in Erlenmeyer flasks of 500 ml, in the same conditions as media and working parameters.

Biomass: 10 g of culture broth were centrifuged at 5000 rpm, 30 min. The deposit was washed with distilled water and brought to the constant weight by drying at 105 °C. The results were expressed in percentage.

Viscosity was determined with two types of instruments:

a) Rotovisko RV1 (Gergruder Haake-Germania). During the determination the material lies between the rotor and the stator of the measuring system. Shear stress is created as a function of the rotation speed at a certain frequency.

The viscosity is measured with the help of the Fourque, which is a function of shear stress and geometry of the measuring system.

The dynamic viscosity is calculated with the formula:

$$\eta_1 = KUS$$

where: $\eta_1$ - the viscosity (Poise or centiPoise); $K$ - Rotovisko RVI constant factor (cP/skt); $U$ - the fixed frequency (without dimension); $S$ - reading on the instrument scale (skt) directly proportional to the torque.

b) Viscosimeter type $B_3$ (VEB Kombinat - Medizin und Labortechnik, Leipzig).

The viscosity is determined by measuring the falling time of a selected ball between the two marks of the apparatus tube.

The dynamic viscosity is calculated with the formula:

$$\eta_2 = t(\rho_1 - \rho_2)k$$

where: $\eta_2$ - the dynamic viscosity (cP); $\rho_1$ - the ball density (g/cm$^3$); $\rho_2$ - the liquid density at working temperature (g/cm$^3$); $t$ - the falling time of the ball (s); $k$ - the ball constant.

## RESULTS AND DISCUSSIONS

The cell mass accumulation in the bioreactor determines a rise of viscosity which gives some pseudoplastic properties to the culture broth.

The increase of broth viscosity worsens the homogeneity conditions, which results in the rise of energy input required for the maintenance of the same agitation level in the bioreactor.

The measured viscosity values of the samples taken from different parts of the bioreactor were hardly reproductible.

These difficulties result from the nature and continous modifications of the chemical and physical properties of the culture broth produced by the mycelium accumulation.

In the same time, zones with different media composition caused by uniform mycelium dispersion and by constructive and technological parameters of the bioreactor are formed.

This phenomenon is more evident in media variants ($V_1$-$V_4$) with dry fermented apple pulp and wheat bran.

In the beginning, these culture media have a pseudoplastic behaviour, their viscosity decreasing as a function of the shear rate of the apparatus rotor used for the assay (Fig. 1).

As it can be seen in the Fig. 1, the curve evolution is perturbed by the influence of the particles from the broth culture. For this reason, the dynamic viscosity could not be determined with the viscosimeter $B_3$ because

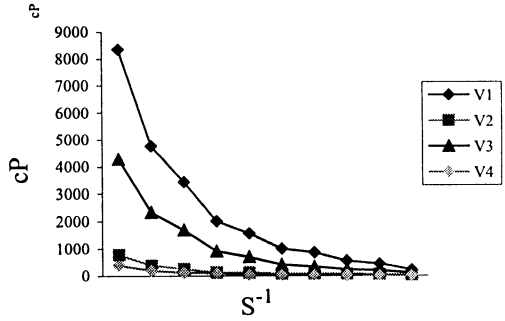


**Fig. 1.** Variants $V_1$ - $V_4$. Viscosity evolution as a function of shear rates.

very often the particles stopped the ball, hindering its movement. For these media, the viscosity is significant only as a function of rotation speed, of shear stress or of shear rate of the instrument used for the determination.

The viscosity curves for the $V_1$-$V_4$ variants, which were obtained with the Rotovisco viscosimeter are presented in Fig. 2 . As can be seen for all the four variants, the viscosity rises in time.

although for some variants ($V_7$ and $V_8$) the enzymatic activities and the biomass quantities were different. For these bioprocess the dynamic viscosities could be determined directly with $B_3$ viscozimeter.

Since the shape of viscosity curves was the same, in all the above mentioned variants, only the $V_8$ variant is represented in Fig. 3.

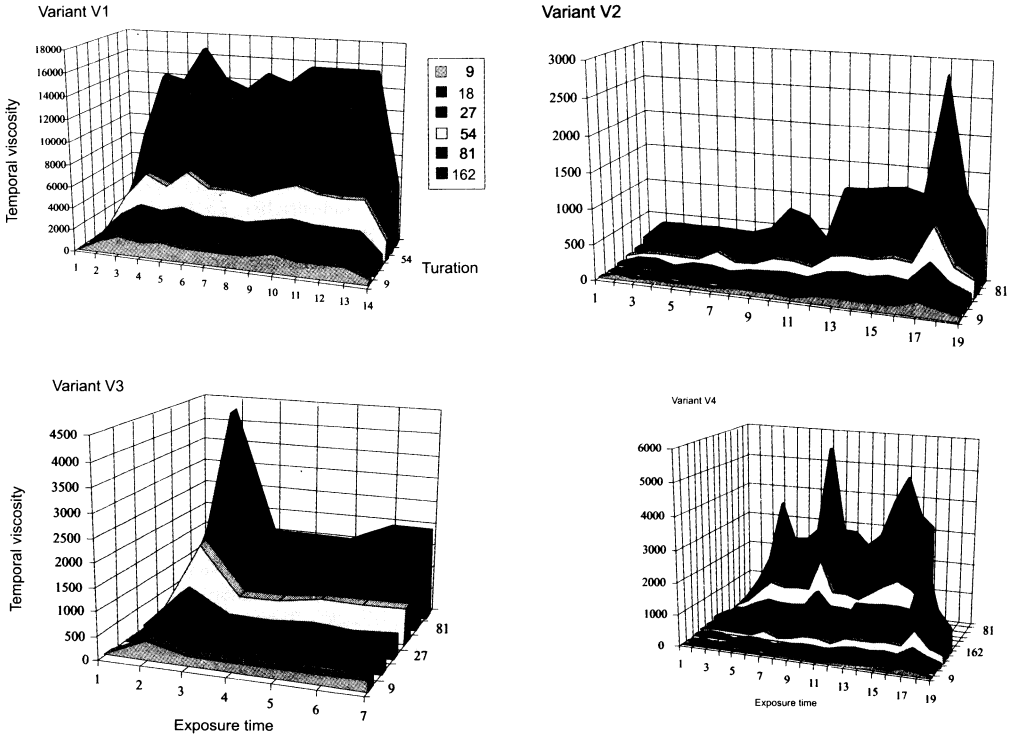


**Fig. 2.** Variants $V_1$-$V_4$. Culture media. Viscosity curve as function of time.

Since parts of the media components, which determine non-newtonian behaviour, are hydrolized and exhausted in time, it is impossible to distinquish whether the determined viscosity is due to the media culture or to the fungi filaments. In order to evaluate the viscosity due to the microorganism development, culture media with saccharides as carbon source have been used (variants $V_5$-$V_8$).

For these media, the initial viscosity was the same, unaffected by the shear stress. These media had also close values of viscosity levels both in the beginning and during the bioprocess,

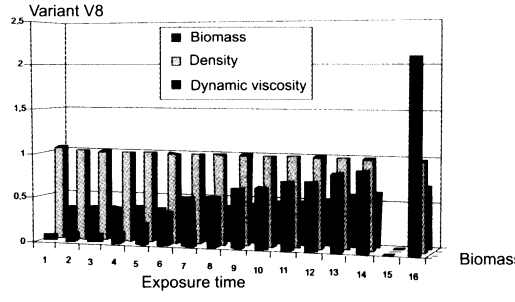


**Fig. 3.** Variant $V_8$. Viscosity, biomass and cdnsity evolution in time in a bioprocess of pectinolytic enzymes obtenance using as carbon source a mixture of mono- and oligosaccharides.

These viscosity levels were measured with the Viscosimeter type $B_3$ (VEB Kombinat - Medizin und Labortechnik, Leipzig) which determines the value of dynamic viscosity as a function of media density and consequently as a function of biomass.

In Figure 3 the time-evolution of viscosity, density and biomass quantity are represented for the bioprocess in the variant $V_8$. It can be observed that the values of viscosity and biomass quantity are increasing in time, while the density is decreasing with time. The media of this type, initially having a newtonian behaviour, become non-newtonian during the mycelium accumulation.

The data from Table 1 and Fig. 3 show that even from the beginning the viscosity of culture media with dry fermented apple pulp and wheat-bran is $10^4$-$10^5$ higher than the viscosity of culture media with saccharides as carbon sources.

The rheological behaviour of any fungi bioprocess requires viscosity and shear stress values for the characterisation and control of such bioprocesses.

## CONCLUSIONS

Results presented in this paper clearly indicate that during bioprocesses in which pectolytic enzymes are obtained with *Aspergillus niger* fungus broth viscosity and its evolution need to be taken into consideration for the analysis of oxygen, energy or mass transfer in the bioprocess.

It is also obvious that culture media with cheap ingredients (dry fermented apple pulp and wheat-bran) are economically advantageous, but they are difficult to characterised. That is because rheological properties vary considerably.

In the studies of mathematical modelling in order to perform a computer assisted bioprocess, data on viscosity evolutions are indispensible.

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