Journal of Microbiology and Biotechnology
Vol. 1, No. 1, 70-74, 1991

Rheological Characteristics of Rifamycin B Fermentation Using *Nocardia mediterranei*

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Received 12 November 1990 / Accepted 14 March 1991

The rheological properties of rifamycin B fermentation broth using *Nocardia mediterranei* were characterized in concomitance with the chemical changes of the fermentation broth. The data were interpreted with various rheological models. As results, it was found that the rheological behaviour at the early growth phases of the culture was the Newtonian fluid, but it was changed to the non-Newtonian fluid (Casson plastic behaviour) at the later phases of the culture. Rheological parameters *viz.*, apparent viscosity (μ_a), yield shear stress (τ_o), and Casson constant (K_c) were changed through the fermentation where cell concentrations were changed and mycelia were greatly branched. Those results indicated that cell concentration and cell morphology played important roles to determine the rheological characters.

Rifamycin B is an antibiotic produced by *Nocardia mediterranei* which produces the antibiotic when cells differentiate to form branched mycelia and spores (6, 15). Fermentor design and the operation conditions are thought to regulate physico-chemical properties of fermentation culture broth. Cell differentiation is affected by the operation conditions which are very determinative for the rheological properties of culture broth (2-4, 12). It has been reported that the on-set of the antibiotic production is closely associated with the cell differentiation. But the highly branched mycelia give rise to increase the viscosity of culture broth and subsequently to interfere with proper mixing and aeration, from which the antibiotic production was greatly reduced (5, 7, 13, 14). These phenomena are thought to be one of the major limitations for the scale-up of antibiotic fermentation process development, therefore, various approaches were attempted to control the viscosity in relation with cell differentiation using various filamentous micro organisms, such as, *Penicillium chrysogenum* and *Streptomyces* spp. (3-6, 8-17, 19).

In this study, rheological properties of rifamycin B fermentation using *N. mediterranei* were characterized with the chemical changes of fermentation broth and the phenomena were analyzed with the cell differentiation.

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Key words: Rifamycin B, rheology, Casson plastic fluld, cell differentiation, morphology

MATERIALS AND METHODS

Microorganism and Maintenance

Microorganism used in the present study was *Nocardia mediterranei* YCK1 which was provided from the Yuhan Chemical Co. Ltd. Vegetative cells grown on a seed culture medium were rapidly frozen by a deep freezer maintained at −70℃ and kept in the freezer until use.

Culture Media

Seed culture medium was formulated as: 2.0% glucose, 0.5% yeast extract, 0.25% enzymatic hydrolyzed casein, and 0.5% malt extract (pH 7.0). Fermentation medium was consisted of 10% glucose, 3.5% soybean meal (200 mesh), 0.8% $(NH_4)_2SO_4$, 0.1% KH_2PO_4, 0.85% $CaCO_3$, 0.08% $MgSO_4 \cdot 7H_2O$, and 0.1% Na-barbiturate (pH 7.0).

Culture Conditions

The frozen cells were melted at ambient temperature, then inoculated to the seed culture medium in baffled flasks and cultured at 28℃ for 2 days using a rotary shaking incubator. The seed culture was inoculated to 3 liters of fermentation medium in a 5 liters culture vessel (Korea Fermentor Co.) where agitation speed and aeration rate were controlled to 500 rpm and 1 vvm, respectively. And culture temperature was maintained at 28℃.

Analytical Methods

Culture broth was taken from the fermentation and centrifuged at 3,000×g for 5 min to seperate cells from fine particles such as soybean meal. The separated mycelia were resuspended in a saline solution to give the original volume using 20 *ml* mass cylinders and then let the mycelia settle down for 20 min. The packed mycelia volume (PMV) was measured and expressed as % to the total volume. The cell free culture broth was recentrifuged at 10,000×g for 10 min and the supernatant was used for the subsequent analyses. Glucose concentration in the culture broth was determined with the dinitrosalicylic acid (DNS) method (8). Ammonium-N concentration in the culture broth was determined by the Van Slyke's method and total nitrogen concentration was analyzed by the Kjeldahl digestion method (9). Rifamycin B concentration was analyzed by determination of adsorption optical density at 425 nm as a reported in elsewhere (10). Dissolved oxygen tension (DOT) was determined using a galvanic electrode. Oxygen transfer rate (OTR) and oxygen uptake rate (OUR) were calculated by the dynamic gassing-out method (18).

Analysis of Rheological Parameters

A rotational spindle viscometer (Brookfield LVF) was used for the determination of shear stresses (τ) and apparent viscosities (μ_a) at different shear rates (γ). The relationship between shear rates and shear stresses can be expressed as Equation 1 for the Newtonian fluid. However when it changed to the non-Newtonian flow, those relations were modelled to the Casson' law as shown in Equation 2 (1). The rheological parameters of the Equation 2 were calculated by regression analysis.

$$\tau = k\gamma \quad (1)$$

$$\sqrt{\tau} = \sqrt{\tau_o} + K_c\sqrt{\gamma} \quad (2)$$

The relationships between shear rates and spindle speeds can be expressed as Equation 3.

$$\gamma = kn \quad (3)$$

In the Newtonian fluid, the shear rates are directly related to the spindle speeds hence the ratio between the shear rate and spindle speed (k in Equation 3) in the Brookfield (Model LVF) representes as 1. However in the non-Newtonian fluid, the k values are not constant and varies with the geometrical dimensions and the types of the viscometers used. Therefore shear rates (γ) in the non-Newtonian fluids are expressed as representative shear rates (γ_{rep}) (11).

RESULTS AND DISCUSSION

Chemical Change in Fermentation Broth

Changes in the concentrations of biomass, glucose, NH_4^+-N, pH, and rifamycin B produced in a batch culture are shown in Fig. 1. It was evident that the utilization of glucose was very slow and repression of the rifamycin B production by glucose was not apparent. The culture pH was closely related to the concentration of ammonium ions. It was clear that cell growth was very strong at an earlier exponential growth phase where ammonium ions were sufficient, on the orther hand, rifamycin B production was greatly repressed. In other words, the production of rifamycin B was initiated where the concentration of ammonia ions was sufficiently low. It was thought that ammonium ions were more important than glucose for the maximal production of rifamycin B.

Specific oxygen uptake rate (OUR) was increased as the cell growth and the initiation of rifamycin production hence dissolved oxygen tension (DOT) was rapidly declined to give the minimum value where the rifamycin production was very active. It was considered therefore that controlling areation and agitation to increase DOT would be very important for the optimization of rifamycin B production in connection with the utilization of carbon and nitrogen sources.

Rheological Properties

The relationships between shear stresses and shear

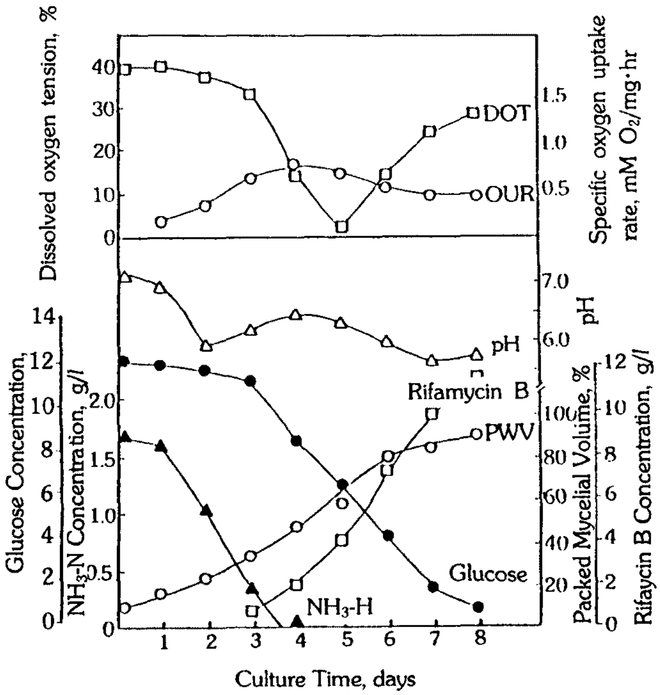

Fig. 1. The time course of rifamycin B fermentation by *Nocardia mediterranei*.

rates were measured with culture broth obtained from different growth phases of the batch culture as shown in Fig. 2, it was evident that linear relationships were obtained with the culture broth of 1~3 days cultivation where cell concentrations were relatively low and cells grew by tip growth to form straight filamentous mycelia but not branched. Therefore, the rheological properties of the 1~3 days cultured broth were thought to be the Newtonian fluid.

However it was interesting to note that the relationships between shear stresses and shear rates were no longer linearly related with the culture broth of 4~7 days cultivation where cell concentrations were increased and cells grew more profoundly branched. It indicated that the rheological behaviour was changed from the Newtonian fluid to non-Newtonian fluid. In this sense, the rheological characters of the culture broth were analyzed with various rheological models proposed (1). As result, it was found that the characters were reasonably fitted to the Casson plastic model as shown in Fig. 3. And rheological parameters *viz.* yield shear stress (τ_o), Casson's constant (K_o) were calculated by regression analysis and apparent visosity was estimated by the Equation 4.

$$\mu_a = \frac{\tau}{\gamma} \qquad (4)$$

As shown in Fig. 4, it was evident that Casson constant (K_c) was increased as the culture time elapsed where the biomass concentration was incresed and cells be-

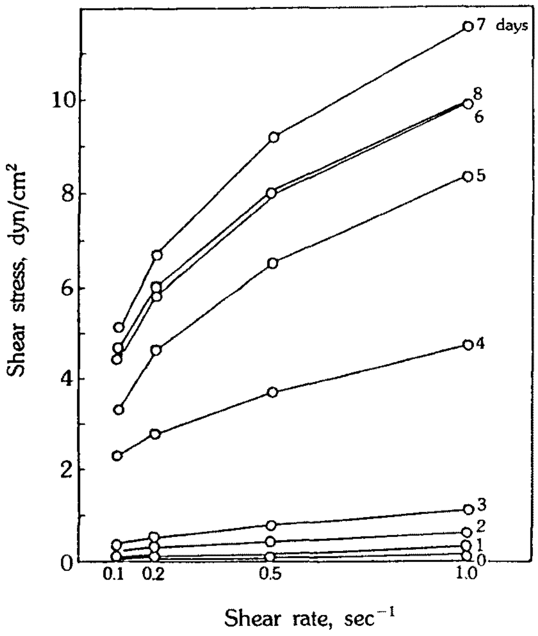

Fig. 2. Shear rate-shear stress relationship for rifamycin B broth during fermentation.

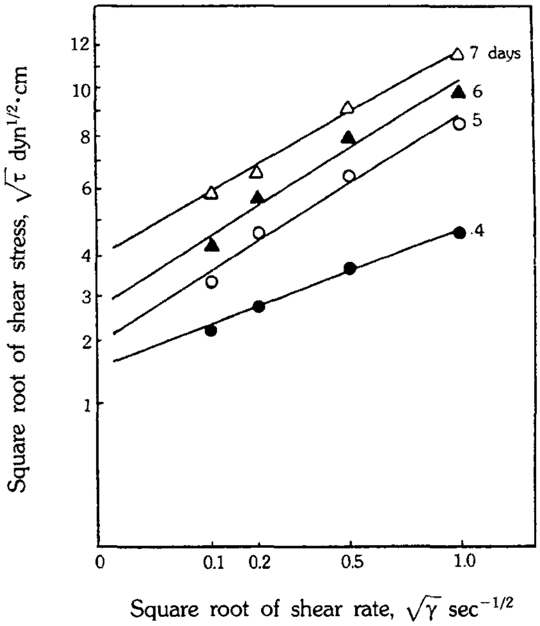

Fig. 3. Analysis of rheological parameters of the Casson's law.

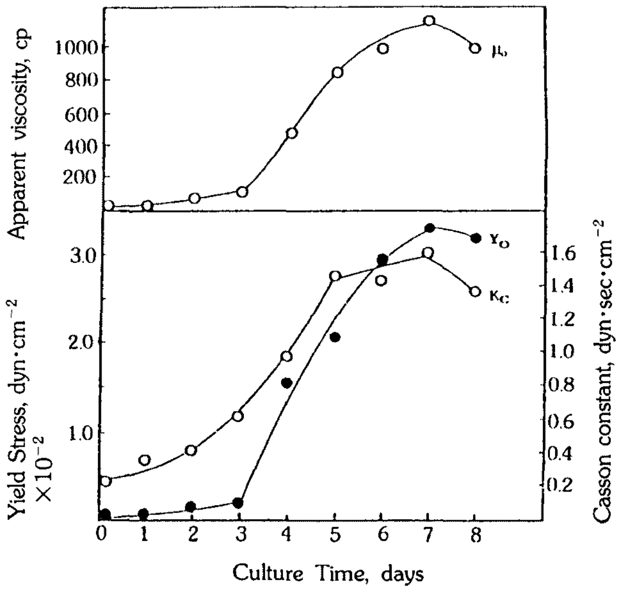

Fig. 4. Changes of rheological parameters of rifamycin B fermentation broth

μ_a: apparent viscosity, cp

Y_o: Yield shear stress, dyn·cm^{-2}

K_c: Casson constant, dyn·sec·cm^{-2}

came more branched (Fig. 5). Furthermore, it was clear also that the apparent viscosity of the culture broths were increased as the culture time elapsed. The results indicated that the culture broth were more resistant to shear force, which might be resulted from the cell concentration and mycelial rigidity.

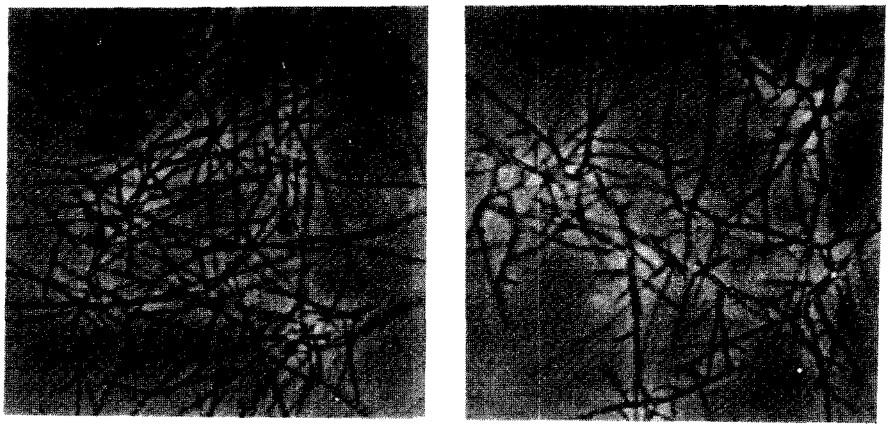

Fig. 5. Microphotograph of mycelial morphology at the tropophase A and the idiophase B.

Furthermore, it was clear that yield shear stresses (τ_o) at the unionculated culture broth were zero and also relatively low at the lag and accelerating growth phases (0~3 days) where the cells were filamentous and tip mode growth was apparent. But those were increased rapidly as the culture time elapsed (after 4 days cultivation) where cells were more branched and formed network like structures at static condition. In other words, cells were so tightly stacked that the higher yield shear stress initiating the motion of flow was required with the higher cell concentration and also highly branched cells (Fig. 5).

$$\tau_o = \delta^* X^a \quad (5)$$

It was reported that the yield stress was functioned both by mycelium concentration and mycelium morphology as given by the Equation 5 (12). The morphological factor (δ^*) of mycelium was characterized by the ratio of cell length and diameter of mycelial. The power values (a) of cell concentration (X) were reported to be 2 at the ideal culture of filamentous fungi and to be 2.5 at the culture of *Pen. chrysogenum* (11). It indicated that the yield shear stress is affected more apparently by mycelia morphological factor rather than by cell concentration. It was interesting that the both values of τ_o and K_c were very closely related to the age of mycelium. The apparent viscosity and the yield shear stress at the late of the culture broth were reduced, which were brought from the apparent fragmentation and autolysis of cells.

From the analyse of the rheological characters, it could be concluded that the rheological behaviour of rifamycin B fermentation broth using *Nocardia mediterranei* would follow the Newtonian fluid behaviour at early part of the fermentation *viz.* 0~3 days, but it changed to the Casson plastic behaviour. It was thought also that the properties were greatly affected by the degree of cell branching rather than concentration of cells. The environmental conditions effected firstly on the morphological characters of cell, which in turn determine the rheological properties of broth. Therefore, it was thought that controlling the rheological properties would be the most important factor to optimize the rifamycin B production using *Nocardia mediterranei*.

Nomenclature

τ = shear stress ($dyn \cdot cm^{-2}$)
τ_o = yield shear stress ($dyn \cdot cm^{-2}$)
γ = shear rate (sec^{-1})
μ_a = apparent viscosity (cP)
K_c = Casson constant ($dy. \cdot sec \cdot cm^{-2}$)
$K_c/\sqrt{\tau_o}$ = Casson's consistency index
X = mycelium concentration (mg/ml)
δ^* = morphological factor which was determined by the ratio of length and diameter of mycelium
k = ratio between the shear rate and spindle speed
n = spindle speed (sec^{-1})

Acknowledgement

The authors are deeply appreciate to Professor Yu-Ryang Pyun for his thoughtful discussions from the start of this experiments and also for the helpful comments on the manuscript.

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