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Morphological and rheological properties of culture broth of *Cephalosporium acremonium* M25

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Abstract

Cephalosporium acremonium is a filamentous microorganism producing cephalosporin C. The morphological differentiation of *C. acremonium* in submerged culture is closely related with the rheological properties of culture broth and production of cephalosporin C. In this study, the rheological and morphological properties of culture broth of *C. acremonium* were investigated. In the seed broths of shake-flask and fermenter culture, the Herschel-Berkley equation was in excellent agreement with experimental results in the whole range of shear rate. In the seed broths of shake-flask culture, morphological differentiation into arthrospores affected to changes of apparent viscosity. But results in the fermenter culture, morphological factors such as mean hyphal thickness and the number of tips gave more effect on changes of apparent viscosity than differentiation into arthrospores. Overall, it suggested that the morphological parameters measured by image analysis can be used as a good parameter to indicate the rheological properties of culture broth of *C. acremonium* M25.

Keywords : *Cephalosporium acremonium*, morphology, image analysis, rheological properties, rheological model, apparent viscosity

1. Introduction

Cephalosporium acremonium, an imperfect fungi, is an object of constant interest on account of its potential use as a producer of cephalosporin and related enzymes. The morphological differentiation of *C. acremonium* in submerged culture is closely related with the production of cephalosporin C (CPC). CPC is a naturally produced -lactam antibiotic which acts against both gram-positive and gram-negative bacteria. It is a starting point in the manufacture of more potent cephalosporins for parenteral use in treating human infections caused by penicillinase-producing bacteria. It was reported that submerged culture of *C. acremonium* in a synthetic medium showed four distinct morphological states in fermentations; hyphae, swollen hyphal fragments (thick, short, and highly septate forms), conidia and germlings (Nash and Huber, 1971). It appears that, in continuous culture, mycelial morphology is influenced by the nature of the limiting nutrient, shear stress and dilution rate. Among those factors, inoculum quality (ie. size, age, and type) is of prime importance in determining the characteristics of the fermentation of filamentous cultures (Lee *et al.*, 2001). Considering inoculum quality, especially inoculum age, the morphology of *C. acremonium* was classified as three types ; hyphae, swollen hyphal fragments and arthrospores. It has been reported that morphological differentiation of hyphae into highly swollen fragments obviously occurred before the onset of CPC production, and highly swollen hyphal fragments gradually differentiated into arthrospores during CPC production (Matsumura *et al.*, 1980). The characterization of the morphology of filamentous microorganisms started years ago (Metz *et al.*, 1981) and, nowadays, methods using fully automated image analysis have been developed for faster and more reliable measurements (Treskatis *et al.*, 1997). Free filaments have been usually described by some parameters such as length of the main hyphae, total hyphal length, number of hyphal tips, and main hyphal thickness. Most of these studies were undertaken to gain an understanding of the growth process and of the relationship between morphology, rheology, and mass transfer, in the hope of finding possible relationships between morphology and secondary metabolite production.

Also the rheological behavior of a fermentation broth is of considerable importance in describing the transport phenomena in the fermenter. The fermentation liquid is actually a suspension of microorganisms which have, in the case of

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mycelial broths, a high ratio of length to diameter (Wittler *et al.*, 1983). From existing suspension theories it is suspected that such suspensions will show, even when the solid content is fairly low, marked non-Newtonian characteristics. An important feature of the batch fermentation process is the change of rheological behavior during fermentation due to the changes in mycelial concentration and morphological characteristics of the mycelia (Lee *et al.*, 1993).

In this study the rheological model applicable for the description of the flow behavior of the seed culture broth was examined and the effect of mycelial morphology on the rheological behavior of the broth was also investigated.

2. Materials and methods

2.1. Strain

The microorganism used in this study was mutant strain, *Cephalosporium acremonium* M25, treated with UV.

2.2. Media and culture conditions

Basal seed medium consisted of 2.5% sucrose, 1.0% glucose, 2.5% corn steep liquor and 0.4% $(NH_4)_2SO_4$. Seed cultures were carried out in 250 m*l* Erlenmeyer flasks containing 50 m*l* of medium. The shake-flask cultures were operated at 300 rpm, 27°C, on a rotary shaking incubator. The batch fermentation in the stirred-tank fermenter (2.5 L, Kobiotech, Korea) was carried out at 27°C. Operating volume was 2.0 L and air flow rate was 1.0 vvm.

2.3. Analysis

2.3.1. Packed mycelium volume (PMV)

In the case of fermentation medium containing insolubles, 10 m*l* samples of culture broth were centrifuged and separated into supernatants and sediments. The volume of

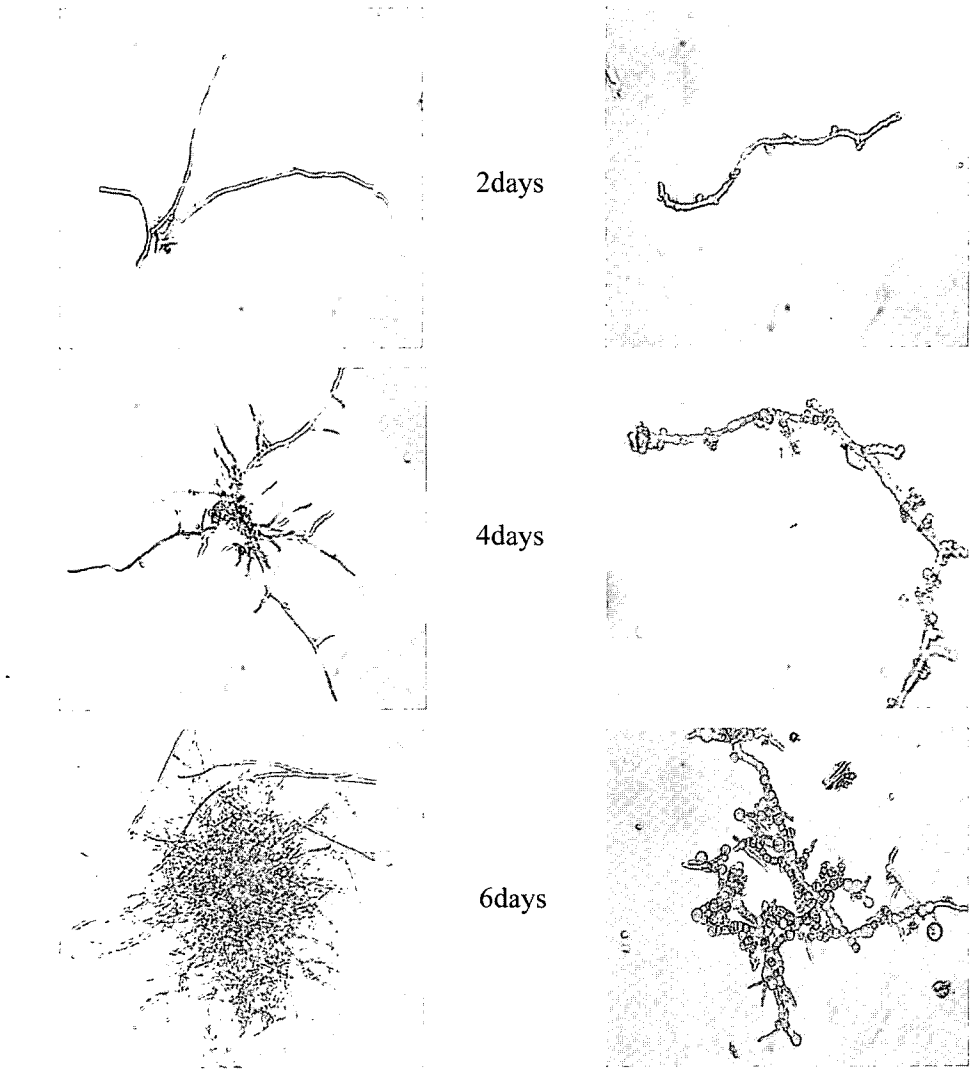


Fig. 1. Typical morphological changes in the shake flask and fermenter cultivations of *C. acremonium*.

cells was expressed as a percentage (Lee *et al.*, 2001).

2.3.2. Image analysis for cell morphology

The cell morphology was studied on photomicrographs with optical microscopy connected with Image Pro 3.0 software.

Morphological factors such as hyphal length, number of tips, number of arthrospores and length of swollen hyphal fragments were measured manually or automatically after sorting and classifying by image analyzing process.

2.3.3. Analysis of rheological parameters

The Brookfield LVT viscometer was used to determine the shear stresses(τ) and apparent viscosities at different shear rates ($\dot{\gamma}^n$). The relationships were interpreted in terms of the different rheological models: Power law model; $\tau = K \cdot \dot{\gamma}^n$, Bingham plastic model; $\tau = \tau_0 + n\dot{\gamma}$ Herschel-Bulkley model; $\tau = \tau_0 + (K \cdot \dot{\gamma}^n)$.

3. Results and Discussion

3.1. Morphological changes of *C. acremonium* M25

Morphological differentiation may have a significant influence on the formation of certain metabolic product; e.g., antibiotics, enzyme, primary metabolites. There have been only few attempts to model fragmentation (or break up) of the mycelium. This is especially important during submerged growth where the mycelium is exposed to shear forces. Fragmentation of the mycelium will result in a formation of individual hyphal elements which may have a varying morphology, e.g., different lengths and number of tips, thus affecting to metabolites production.

Typical morphological changes during 6 days in the seed cultures were taken by a camera mounted on an optical microscope (Fig. 1). Morphological characteristics in the shake-flask and fermenter cultivations show completely different tendency in differentiation. In the shake-flask cultivation, the 2-day seed broth contained many filamentous hyphae and a few swollen hyphal fragments, but no arthrospore. But, in the fermenter cultivation, there were many swollen hyphal fragments and the progress of morphological differentiation was faster than in shake-flask cultivation. In the 4-day seed broth, some filamentous hyphae and large number of swollen hyphal fragments were observed. Swollen hyphal fragments in the fermenter cultivation were much thicker than in the shake-flask cultivation. There were also many swollen hyphal fragments and arthrospores in the 6-day seed broth. By image analysis using digital camera and Image Pro program, morphological factors were measured quantitatively (Fig. 2 and Fig. 3). In the seed broth of shake flask culture, hyphal length was shortened from 70 μm to 10 μm, and the number of arthrospores increased to 1.2×10^2/m*l* during the 6 days. Mean hyphal thickness was increased to 4.05 μm during the 5 days but decreased to 2.95 μm at 6 days. In the seed broth of fermenter culture, hyphal length was lengthened gradually to 395 μm during 6 days. The number of arthrospores increased to 4.55×10^2/m*l* during the 6 days. On the contrary to shake flask culture, the number of tips were rapidly increased after 4 days. However mean hyphal thickness showed same trend as shake flask culture. In the fermenter culture, it was thought that fast differentiation was caused by high oxygen transfer rate and better mixing compared to shake-flask culture.

3.2. Rheological model applicable to the mycelial broth of *C. acremonium* M25

Figs. 4, 5 and 6 show the shear stress versus shear rate plots made by different rheological equations expressing the flow behavior of fermented broths. Fig. 4(a) shows that

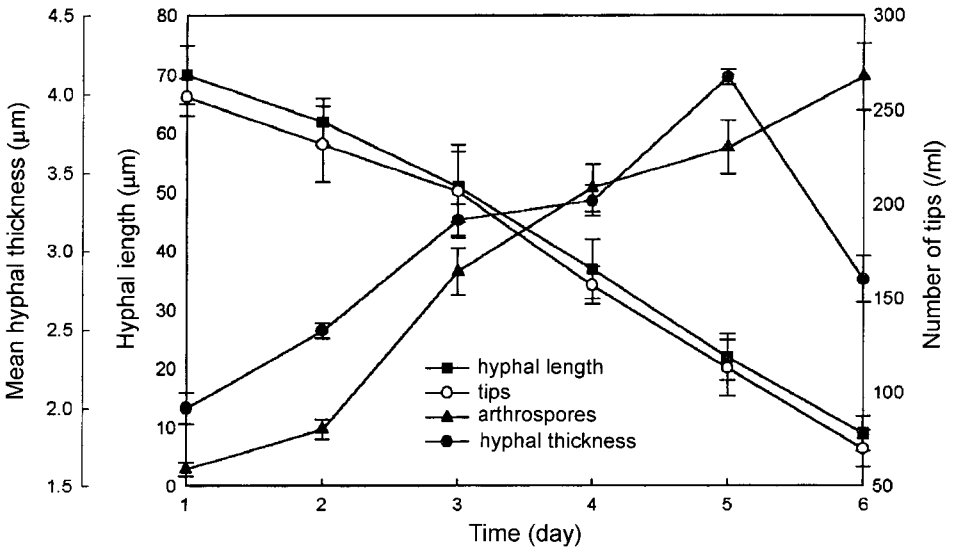


Fig. 2. Time courses of morphological factors of *C. acremonium* M25 in the shake flask culture.

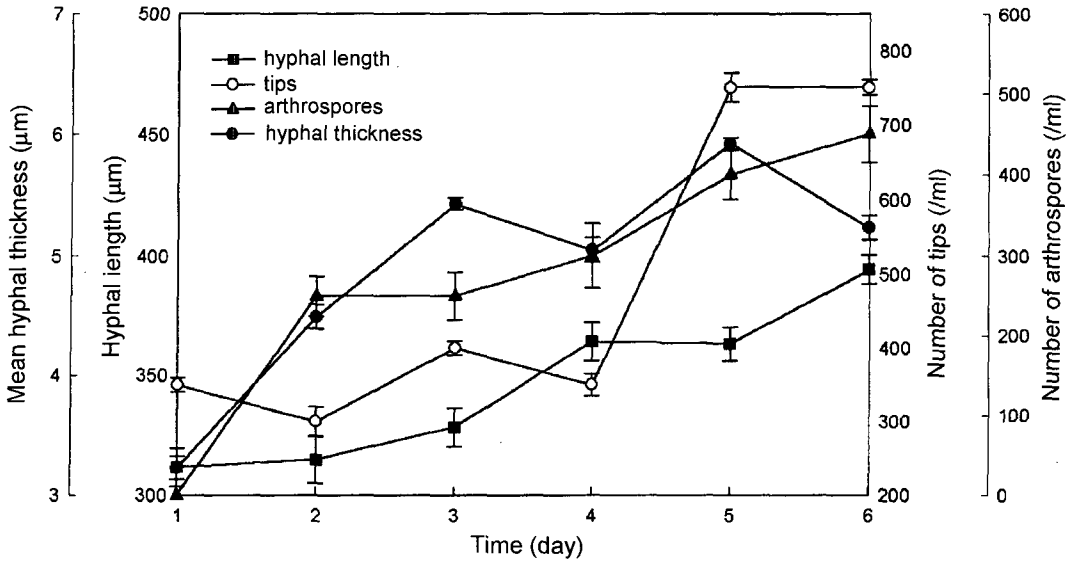


Fig. 3. Time courses of morphological factors of *C. acremonium* M25 in the fermenter culture.

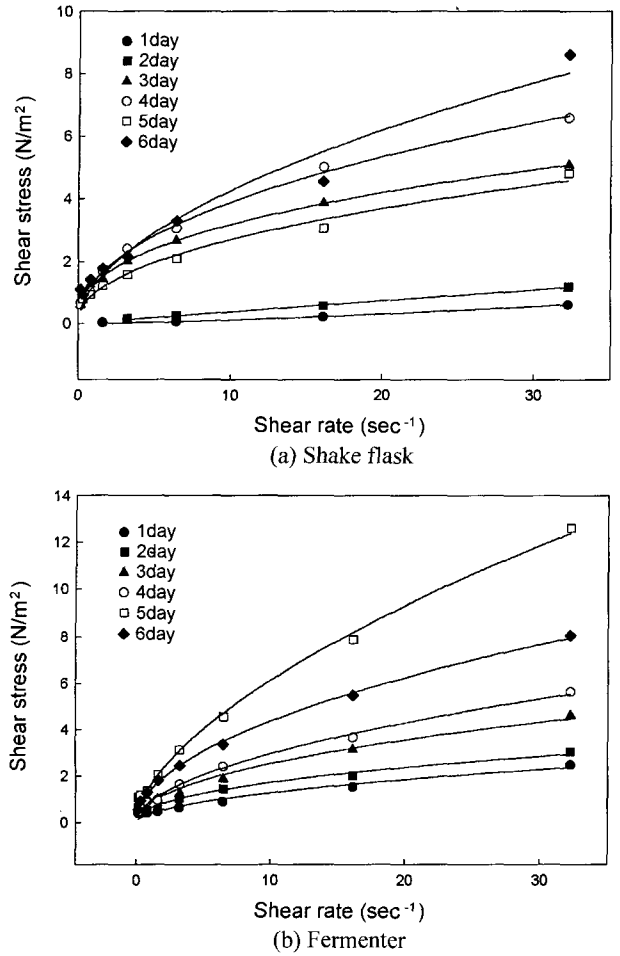


Fig. 4. Shear stress vs. shear rate plots made by the power law equation for the expression of the flow behavior of seed broth at different cultivation time.

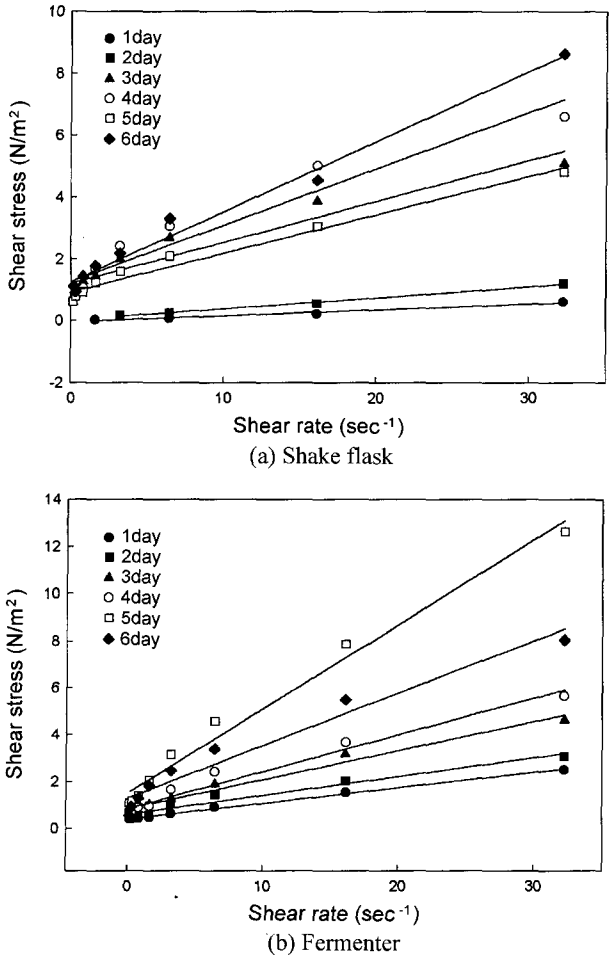
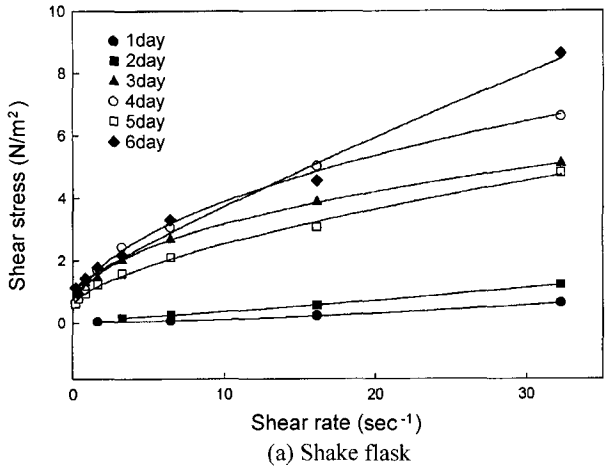
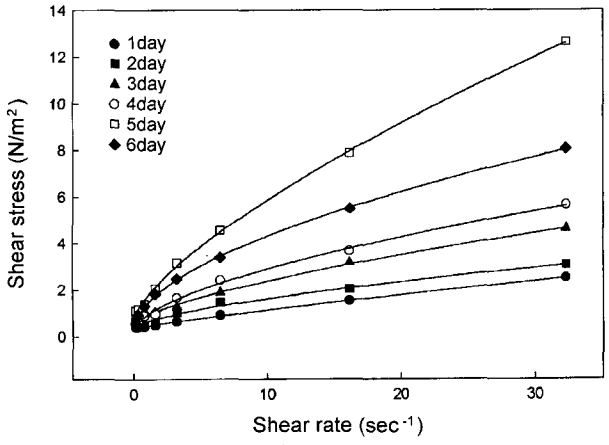


Fig. 5. Shear stress vs. shear rate plots made by the Bingham plastic equation for the expression of the flow behavior of seed broth at different cultivation time.



(a) Shake flask



(b) Fermenter

Fig. 6. Shear stress vs. shear rate plots made by the Herschel-bulkley equation for the expression of the flow behavior of seed broth at different cultivation time.

the experimental results of 6-day broth agree poorly with power law. On the other hand, Fig. 4 (b) shows that the experimental results of 1-3day broths agree well with power law. According to the Bingham plastic equation, it shows a large deviation at the whole range of shear rate tested (Fig. 5). Herschel-Berkley equation is in excellent agreement with result of culture broth from shake-flask and fermenter in the whole range of shear rate (Fig. 6). In the seed broth of shake-flask culture, yield stress was exhibited from 3 days culture broth (Fig. 6 (a)). In the seed broth of the fermenter culture, consistency index increased with the fermentation time. Yield stress tends to be unchanged during the fermentation time (Fig. 7).

In general, flow behavior of culture broth is affected by type of organism used, media, and various culture conditions. In this study, it was expected that mycelial morphology is the most important factor affecting the flow behavior of culture broth. So, relationship between morphology and rheological properties of the culture broth was investigated.

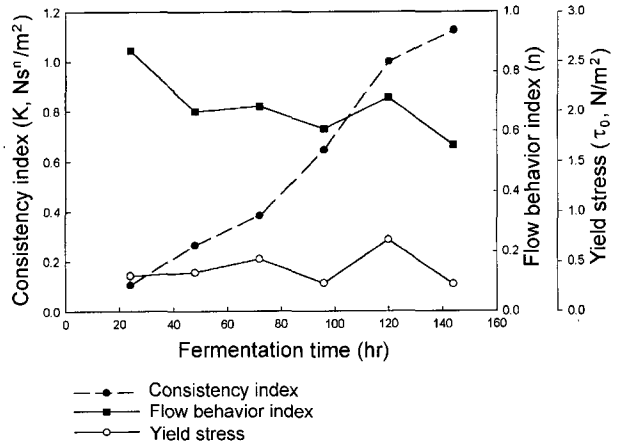


Fig. 7. Changes in flow behavior index, consistency index and yield stress of the seed broth in the fermenter culture.

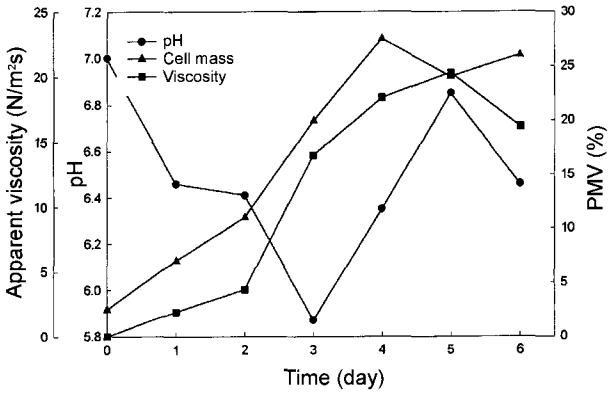


Fig. 8. Seed culture profile of *C. acremonium* M25 in the shake flask.

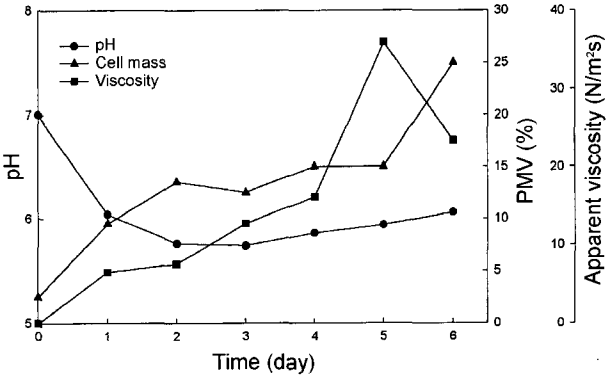


Fig. 9. Seed culture profile of *C. acremonium* M25 in the fermenter.

3.3. Effect of mycelial morphology on the rheological behavior of the broth

Cell mass, pH, and apparent viscosity were shown in growth curves of *C. acremonium* in the shake flask (Fig. 8) and in the fermenter (Fig. 9). PMV was increased to 27%

during the 4 days, and then decreased finally to 26% in the seed broth shake flask culture. Apparent viscosity showed similar tendency to cell mass. But it decreased rapidly after 5 days. It is assumed that the differentiation of swollen hyphal fragments into arthrospores caused the decrease of apparent viscosity. In the seed broth of fermenter culture, PMV was increased gradually to 15% during the 5 days, and then it increased rapidly to 25% at 6 days. Apparent viscosity increased gradually to 16.5 Pa.sec during the 4 days. Then it increased rapidly up to 35.9 Pa.sec at 5 days, and decreased rapidly at 6 days. In this case, it is assumed that morphological factors such as mean hyphal thickness and the number of tips gave more effect on changes of apparent viscosity than differentiation into arthrospores.

4. Conclusion

From the analysis of the morphological characteristics, seed broths from shake-flask and fermenter cultures showed very different morphological pattern. It is therefore thought that different culture conditions could affect to the morphology, thus rheological properties. In the analysis of rheological model applicable to the seed broth, Herschel-Berkley equation was in excellent agreement with result of culture broth from shake-flask and fermenter. In the seed broths of shake-flask culture, morphological differentiation into arthrospores affected to changes of apparent viscosity. But, in the seed broths of fermenter cultivation, morphological factors such as mean hyphal thickness and the number of tips gave more effect on changes of apparent viscosity than differentiation into arthrospores. Overall, it suggested that the morphological parameters measured by image analysis can be used as a good parameter to indicate the rheological properties of culture broth of *C. acremonium* M25.

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