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Effect of Antifoam Agents on Δ^1-Dehydrogenation of Hydrocortisone

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Hydrocortisone 의 Δ^1-Dehydrogenation 에서 소포제의 영향

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Effect of antifoam agents, silicone oil and neolin 302, was investigated on the production of prednisolone by microbial Δ^1-dehydrogenation of hydrocortisone. The microbial process was conducted by using a pseudo-crystallofermentation. By the hydrophobic-hydrophobic interaction, the steroid crystals aggregated with the antifoam agents. The aggregation resulted in a decrease of total mass transter area of substrate particles which is proportional to the dissolution rate of the solid substrate, and it consequently led to a significant decrease of the bioconversion rate. The bioconversion with neolin proceeded more slowly than with silicone oil. Increase of the concentration of the antifoam agents also yielded a significant decrease of the bioconversion rate.

Δ^1-Dehydrogenation of hydrocortisone by *Arthrobacter simplex* is an important and very practical conversion process to produce prednisolone. It was found quite early (1954) during the period of intensive research on steroids that the introduction of a 1, 2 double bond into steroids such as cortisone or a 1,2 doudrocortisone formed products (prednisone and prednisolone, respectively) that had 3 to 5 times the potency of their precursors. In addition, side effects such as retention in humans were greatly diminished (1).

Because of its hydrophobic nature, hydrocortisone has a low aqueous solubility which generally leads to diffusional limitations in the reaction mixture. Therefore, a pseudo-crystallofermentation process in which fermentation proceeds in a heterogeneous phase has been suggested to avoid these drawbacks (2-4). This process is thus being run at considerably higher substrate levels in order to achieve acceptable economics.

Although a pseudo-crystallofermentation is the only microbial process that is being adopted to produce steroids on a large scale, the basic data such as the optimized culture conditions and fermentation parameters are hardly revealed probably because the industry are not willing to release their process data and techniques. There have been many reports on the microbial Δ^1-dehydrogenenation of hydrocortisone, but they have focused on the academicresearch rather than the practical applications (5-10). Recently, the authors have reported the practically usable data obtained from the study on the effect of fermentation parameters in Δ^1-dehydrogenenation of hydrocortisone (11). It was also noted that use of the anifoam agents in the fermentation system resulted in a significant decrease in the overall bioconversion rate. In the present study, we have observed that the agreegation of steroid crystals, which was further facilitated by the antifoam agents, greatly influence the bioconversion rate, and herewith we report the results.

Key words: Δ^1-Dehydrogenetion, pseudo-crystallofermentation, antifoam agent, dissolution rate
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Materials and Methods

Microorganism

Arthrobacter simplex (ATCC 6946) was used throughout this study.

Chemicals

Hydrocortisone and prednisolone were obtained from Prodotti Gianni (Milano, Italy) and analytical reagents such as chloroform, methanol, glacial acetic acid and ethyl acetate were all HPLC grade. Sillicone oil and neolin 302 were obtained from Sigma Chemicals (U.S.A.) and Korea Polyol Co. (Korea), respectively.

Culture conditions

A. simplex was cultivated in a medium containing glucose (2%), yeast extract (1%), KH_2PO_4 (0.1%), K_2HPO_4 (0.1%). The medium was adjusted to pH 7.3 with 2N NaOH. The seed culture was grown on a shaking incubator (Korea Scientific Instrument Co., Seoul, Korea) for 20 hrs at 30°C (160-180 rpm).

Bioconversion

Bioconversion was carried out in a 500 ml Erlenmyer flask containing 100 ml liquid medium at 160-180 rpm and 35°C. The medium used in the bioconversion consisted of casamino acid 0.2%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $MgCl_2$0.001%. The pH was adjusted to 7.3 with 2N NaOH before autoclave. Hydrocortisone at a concentration of 50 g/L was used as the substrate in a crystalline state. It was added to the fermentation broth containing growing cells and the antifoam agents, and then converted to prednisolone.

Analytical methods

The samples from the bioconversion experiment were immediately treated with ethyl acetate to extract steroids. The ethyl acetate phase than subjected to HPLC (Hitachi Model 665a-12, Tokyo, Japan) analyses for the detection of hydrocortisone and prednisolone, as described earlier (11).

Results and Discussion

As already reported in the previous study (11), severe foaming occurred in the jar fermentor operation on adding the hydrophobic substrate crystals to the fermentation broth, and so it was inevitable to use the considerabe antifoam agents at the initial stage of fermentation. Therefore, the effect of antifoam agents on the microbial conversion of hydrocortisone using a pseudo-crystallofermentation technique was investigated through the shake flask experiments.

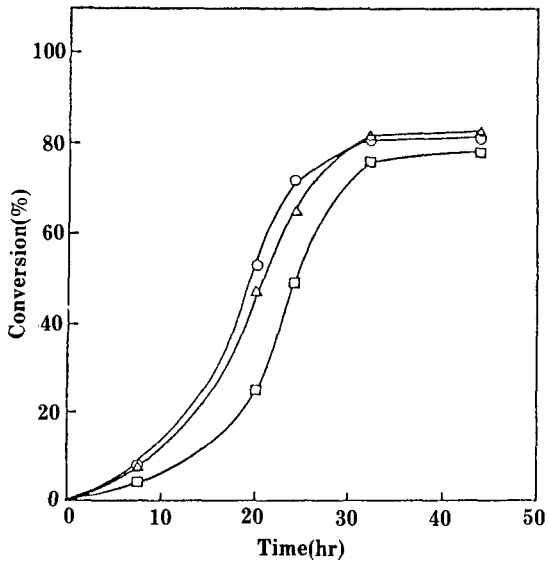

Fig. 1. Effect of the antifoam agents (0.5% v/v) on Δ^1-dehydrogenation of hydrocortisone.
control (○); silicone oil (△); neolin 302 (□)

Fig. 1 shows the effect of antifoam agents on the Δ^1-dehydrogenation of hydrocortisone. Two kinds of anifoam agents, neolin 302 and silicone oil, at a concentration of 0.5% (v/v) were tested and their effects on the bioconversion were compared to the cases without the antifoam agents. Use of the antifoam agents resulted in a decrease of the bioconversion rate. The bioconversion rate with silicone oil was higher than that with neolin. The bioconversion without the antifoam agents reached a stationary phase in 25 hour while stationary phases with silicone oil and neolin were attained in 32 and 44 hour, respectively. Hydrocortisone was converted to prednisolone with a final conversion of ca. 80%., which incomplete conversion was caused by the formation of mixed crystal consisted of 10-20% of the solid substrate, hydrocortisone, lowering the purity of the fermentation product.

The effect of concentration of antifoam agents,

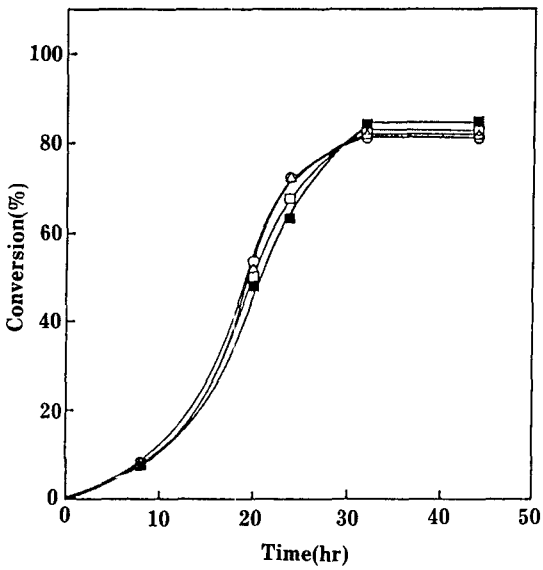

Fig. 2. Effect of the silicone oil concentration on Δ^1-dehydrogenation of hydrocortisone.
control (○); 0.1% v/v (△); 0.25% v/v (□); 0.5% v/v (■)

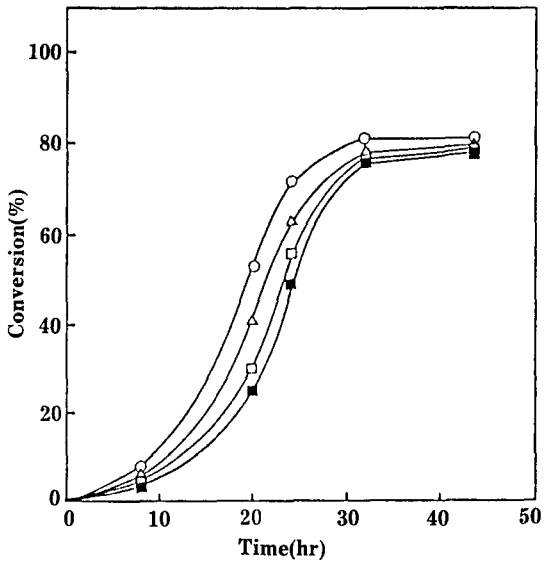

Fig. 3. Effect of the neolin 302 concentration on Δ^1-dehydrogenation of hydrocortisone.
control (○); 0.1% v/v (△); 0.25% v/v (□); 0.5% v/v (■)

silicone oil and neolin 302, is shown in Fig. 2 and 3, respectively. Increase of the concentration of antifoam agents resulted in a decrease of the bioconversion rete and at the same concentration neolin yielded a further decrease of bioconversion rate compared to silicone oil. The bioconversion rate was not affected by the use of silicone oil at a concentration of 0.1% (v/v), but a slight decrease of the bioconversion rate was observed at a concentration of 0.5 (% v/v). In a jar fermentor operation, the antifoam agents at a concentration of ca. 0.5 (% v/v) were used to reduce foaming.

Fig. 4 shows the effect of silicone oil and neolin 302 on the cell growth. It was observed that the use of the antifoam agents at a concentration of 0.5% (v/v) did not affect the cell growth rate. Therefore, it is most probable that the antifoam agents have no direct influence on the cell physiology if they are used within an appropriate level.

A brief remark explaining why the antifoam agents affect the bioconversion rate was made in the previous study (11). It was suggested that the change of particle size induced by the antifoam agents might cause a decrease of the bioconversion rate. More detailed and solid explanations supporting such conclusion were presented in this study.

Fig. 5 shows the change of shape and size of the

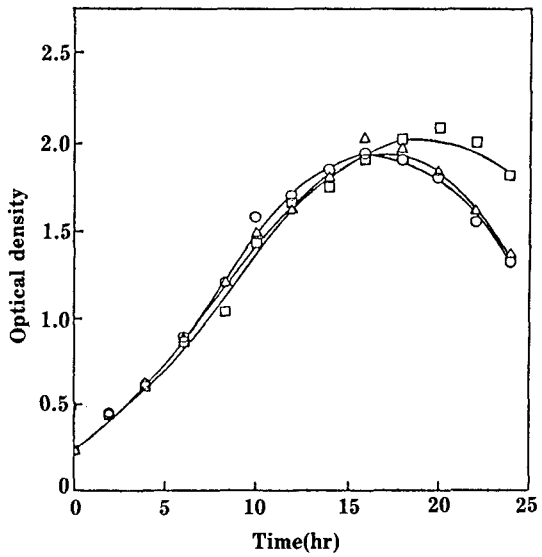

Fig. 4. Effect of the antifoam agents (0.5% v/v) on the growth rate of *A. simplex*.
control (○); silicone oil (△); neolin 302 (□)

particles as the bioconversion proceeded with or without the antifoam agents. Hydrocortisone of a irregular shape was converted to prednisolone of a needle shape by *A. simples* possenssing Δ^1-dehydroenase activity. It was observed that the hydrocortisone particles were aggregated in the presence of the antifoam agents. The agents of aggre-

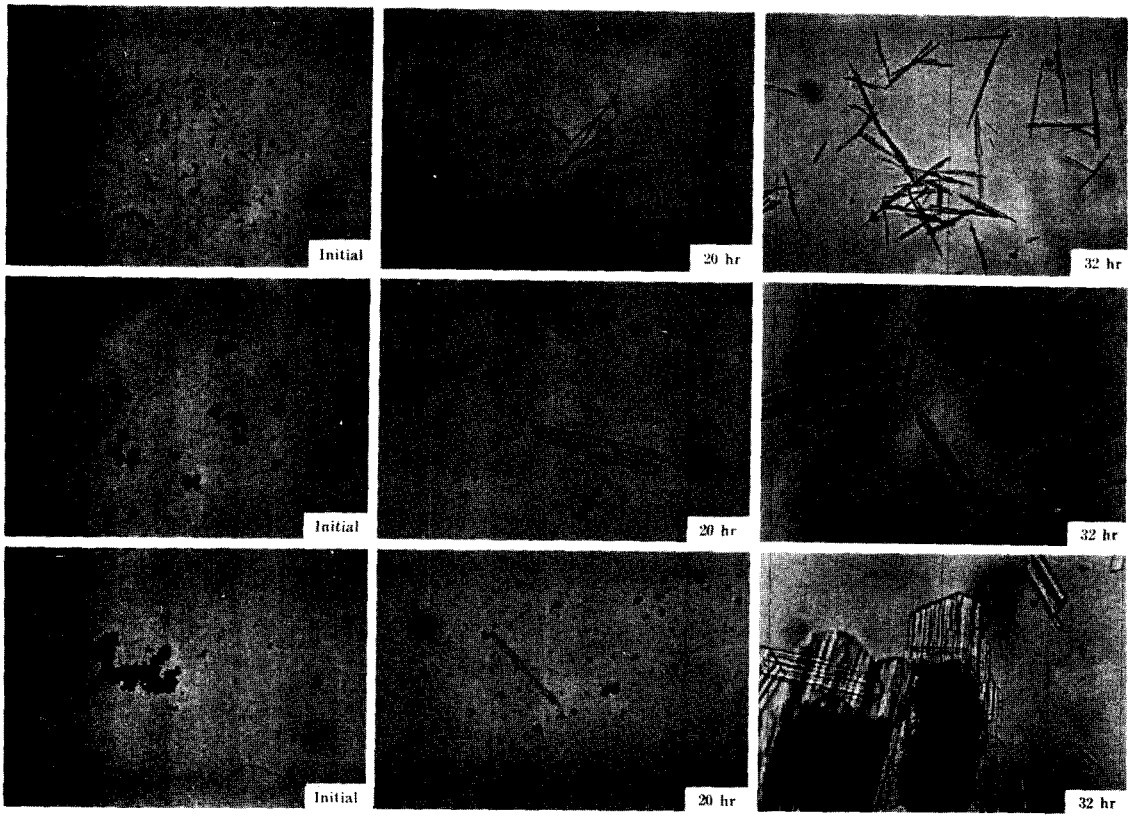

Fig. 5. Microphotographs of the hydrocortisone and prednisolone crystals during the bioconversion (Magnification; x400).
control (upper); silicone oil (middle); neolin 302 (lower)

gation was greater in the case of neolin than silicone oil. The bioconversion proceeded in the state of aggregation and so bigger prednisolone particles were obtained. Fig. 6 shows the effect of neolin 302 concentration on the aggregation of hydrocortisone crystals. As shown in this figure, the extent of aggregation increased with a increase of neolin 302 concentration. It was believed that steroids were aggregated around the antifoam agents dispersed in the fermentation broth because of their hydrophobic-hydrophobic interactions.

The rate of dissolution of solids can be expressed by the product of the mass transfer coefficient (K), the total mass transfer area (A), and the concentration gradient of the substrate in solution (S* - S)(12, 13):

$$\frac{d\bar{S}}{dt} = -kA(S^* - S)$$

In this equation $\bar{S}$ is the concentration of the solid substrate, S* is the solubility of the substrate, and S represents the concentration of substrate in solution. The total mass transfer area (A) is inversely proportional to the extent of aggregation. Therefore, the aggregation of hydrocortisone particles resulted in a decrease of A, which consequently led to a decrease of the dissolution rate. It may be suggested that a pseudo crystallofermentation should be affected by other factors such as the volumetric oxygen transfer coefficient affected by the antifoam agents (14). However, it should be quite reasonable to conclude as a main factor that use of the antifoam agents in Δ^1-dehydrogenation of hydrocortisone results in a decrease of the biocoversion rate because bigger solid aggregates are formed by the hydrophobic-hydrophobic interactions.

Therefore, in Δ^1-dehydrogenation of hydrocortisone by the pseudo-crystallofermentation process,

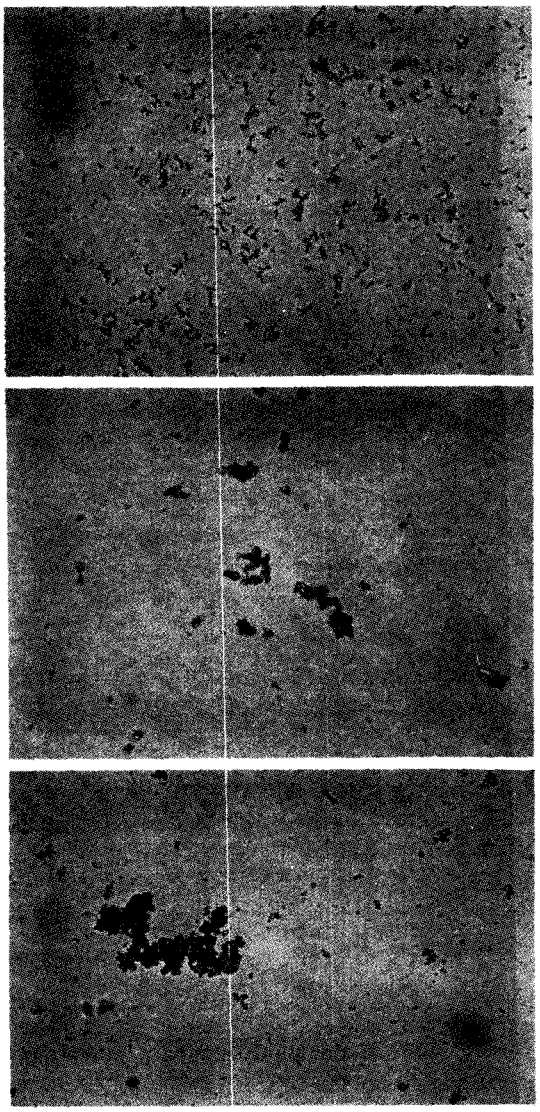

Fig. 6. Microphotographs showing the effect of the neolin 302 concentration on the aggregation of hydrocortisone crystals (Magnification; x400).
control (upper); 0.25% v/v (middle); 0.5% v/v (lower)

the antifoam agents must be carefully selected so that the overall bioconversion rate should not be seriously affected.

요 약

Hydrocortisone의 Δ^1-dehydrogenation에 의한 prednisolone의 생산에서 소포제인 silicone oil과 neolin 302의 영향이 조사되었다. 미생물 전환방법은 pseudo-crystallofermentation 기법에 의해 수행되었다. 스테로이드 입자들은 소포제와의 hydrophobic-hydrophobic interaction에 의해 서로 응집되었다. 이러한 응집 현상에 의해 고체 기질의 용해속도와 비례하는 물질전달 면적이 감소됨으로써, 결국 생물전환속도의 감소를 유발하게 된다. Neolin은 silicone oil에 비해 전환속도에 더욱 좋지 않은 영향을 끼쳤으며, 소포제 농도가 증가할수록 전환속도는 감소하였다.

Nomenclature

A : surface area of substrate particles, (cm^2)
K : mass transfer doefficient ($1/h\ cm^2$)
S : concentration of substrate in solution (g/L)
S*: saturation concentration of substrate (solubility of substrate) (g/L)
$\bar{S}$:concentration of solid substrate (g/L)
t : time (h)

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