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A Study on the Conditions of Demethyltetracycline Fermentation

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Demethyltetracycline 발효조건에 관한 연구

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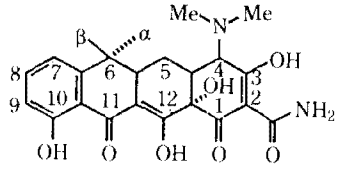
Abstract — Conditions of fermentation for the production of demethyltetracycline were examined using the mutant, which was obtained through the cell fusion of demeclocycline producing strains. The optimum temperature and the initial pH of broth for demethyltetracycline fermentation were 25℃ and 6.7, respectively. Unlike any other cases, the control of pH with alkali solution during the fermentation process affected the productivity. As a general rule, the larger the inoculum size the higher the early consumption of sugar and the viscosity of broth, which means that fermentation proceeds more rapidly as the inoculum size is increased. The highest productivity was shown when the inoculum size was 5% (v/v), and the phase of seed also influenced the fermentation. Among the parameters of pre-culture thus examined, pH was the most important factor.

Demethyltetracycline (DMT) is of small quantity in the fermentation broth of demeclocycline (1) (DMCT). DMT is different from DMCT in the 7-position of molecular structure (Fig. 1), and it has weak antibacterial activity and consequently little value as a drug. DMCT which can be used as an antibiotic due to its antibacterial activity, also has higher value as a precursor in the chemical synthesis of minocycline. In spite of its weak antibacterial activity, DMT can be used in the minocycline synthesis as an intermediate. In the chemical synthesis of minocycline, chloride of 7-position of DMCT must be eliminated, but this step is not necessary when DMT is used as a precursor. This fact makes DMT more valuable in the minocycline synthesis. Little is known about the strains producing DMT dominantly and about the fermentation process, except that content of DMT is 41% of the whole derivatives of DMCT produced by DMCT fermentation (1). We recently isolated a DMT producing mutant through the protoplast fusion which produces DMT up to more than 70% of whole derivatives. Therefore, the conditions of fermentation for this strain were investigated.



	5	6α	6β	7
Tetracycline	H	CH_3	OH	H
Chlortetracycline	H	CH_3	OH	Cl
6-Demethyltetracycline	H	H	OH	H
6-Demethylchlortetracycline	H	H	OH	Cl
Oxytetracycline	OH	CH_3	OH	H
6-Deoxyoxytetracycline	OH	CH_3	H	H
Minocycline	H	H	H	$N(CH_3)_2$

Fig. 1. Structural formula for the major tetracyclines.

Materials and Methods

Strain

Streptomyces aureofacines FUS11-4, which produ-

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ces DMT dominantly, was used (2).

Seed culture

Spores were harvested from a slant to be 10^7 spores/m*l*. 1 m*l* of the spore suspension was inoculated into 50 m*l* of the first seed medium (containing CSL 40 g/*l*, $(NH_4)_2SO_4$ 2 g/*l* $CaCO_3$ 12 g/*l* and rice bran oil 10 g/*l*), and the cultivation was performed at 28℃ for 30~35 hours. 1% (v/v) of this cultivated broth was inoculated again to the second medium, whose composition was the same as the first except for the addition of 5 g/*l* of proflo. The seed-culture was performed at 28℃ for 23~27 hours.

Main fermentation

The inoculum size of the main culture was 5% (v/v), and the medium composition was as follows; corn starch 55 g/*l*, proflo 20 g/*l*, corn steep liquor 5 g/*l*, ammonium chloride 1 g/*l*, casein 3 g/*l*, dry yeast 1 g/*l*, yeast extract 0.3 g/*l*, magnesium sulfate 0.5 g/*l*, sodium chloride 0.5 g/*l*, cupric sulfate 0.05 g/*l*, ferrous sulfate 0.05 g/*l*, citric acid 0.1 g/*l* and benzylthiocyanate 0.001 g/*l*. The temperature and the aeration rate for main fermentation were 25℃ and 1.0 vvm, respectively. Concentration of the dissolved oxygen was maintained to 30% by controlling agitation speed. The culture was carried out for 140~160 hours in the 19*l* fermentor (Bioengineering Model NLF 22, Switzerland).

Assay

Concentrations of DMT, total sugar, ammonium ion, viscosity of broth, and packed mycelium volume (PMV) were assayed according to the methods of Shin (3) and Jahng (4).

Results and Discussion

Effect of temperature on the cell growth and DMT production

Fermentation was carried out under the different temperatures. Both the consumption rate of sugar and the viscosity of broth increased as the temperature rose, which indicated that primary metabolism was prevalent in higher temperatures. The final concentration of DMT produced was maximal when the temperature was 25℃ (Table 1).

Table 1. Effect of temperature on the DMT fermentation

Temperature (℃)	Average volumetric sugar consumption rate (g/*l* hr)	Max. viscosity (arbitrary unit)	DMT potency (mcg/m*l*)
25	1.564	17	1,981
28	1.760	23	1,573
30	1.800	39	1,197

Effect of pH on the DMT production

Initial pH of fermentation medium was adjusted to 6.3-7.0. In this range, cell mass showed little difference, but DMT production was maximal with

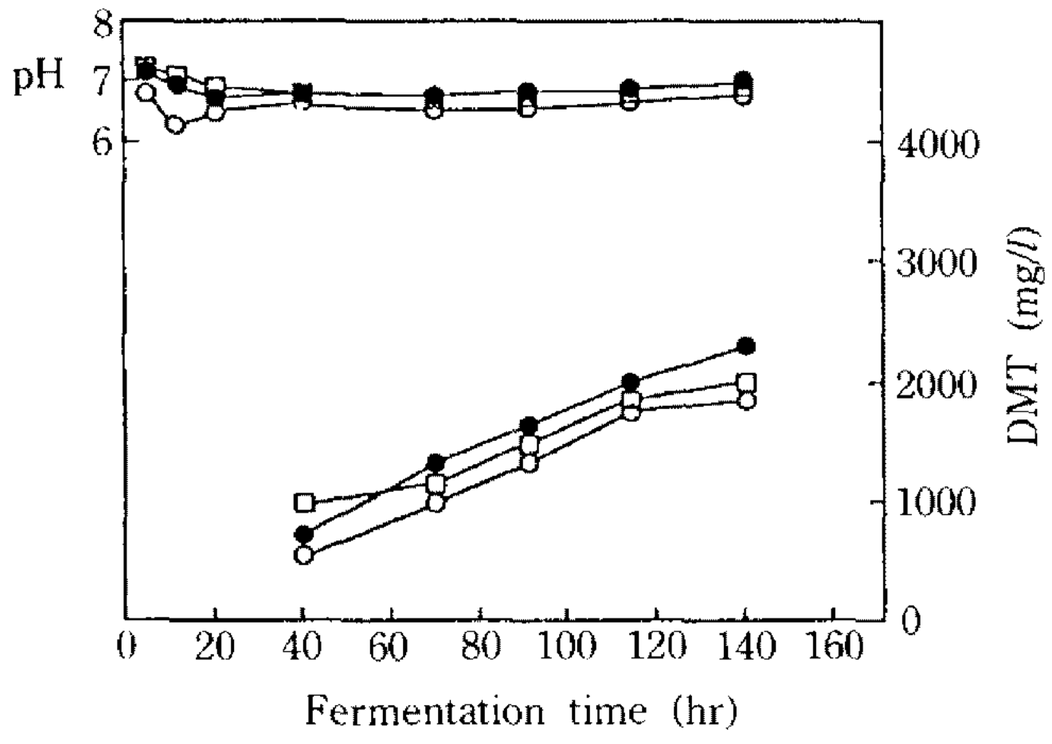

Fig. 2. Effect of initial pH of medium on DMT production.

○-○: pH 6.3, ●-●: pH 6.7, □-□: pH 7.0

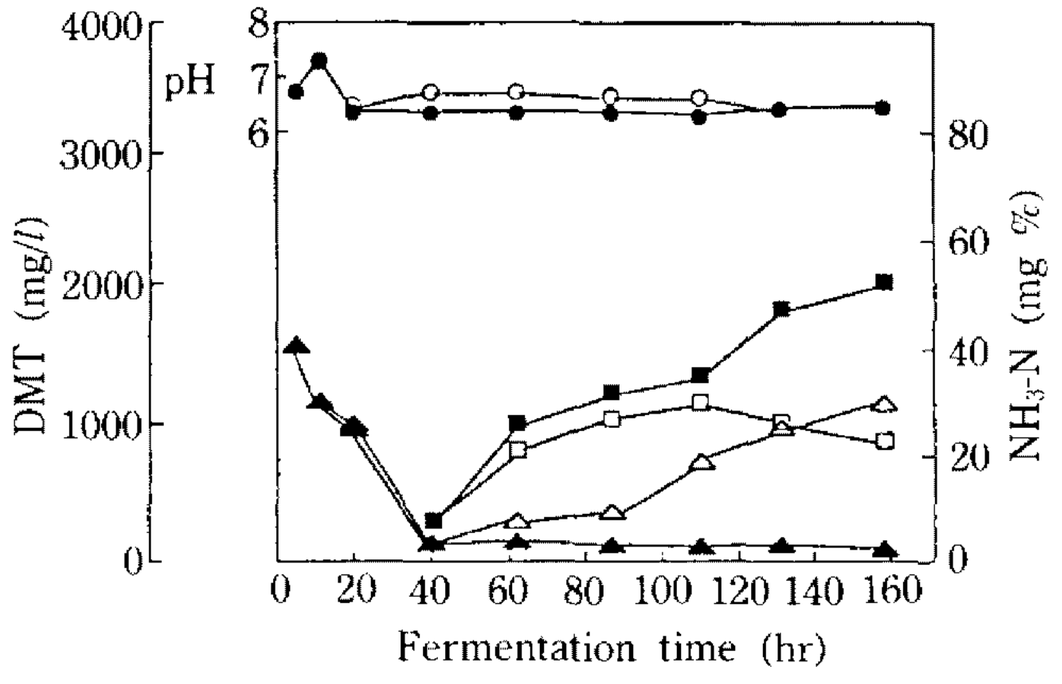

Fig. 3. Effect of pH control on DMT production.

●-●: pH, nature ○-○: pH, control

▲-▲: NH_3-N, nature △-△: NH_3-N, control

■-■: DMT, nature □-□: pH, DMT, control

Table 2. Effect of inoculum size on sugar consumption and viscosity of broth

Inoculum size (v/v %)	Sugar consumed (g/*l*)		Max. viscosity (arbitrary unit)
	0-41 hr	42-65 hr	
5	2.05	1.70	32
7	2.30	1.65	36
10	2.97	0.38	40

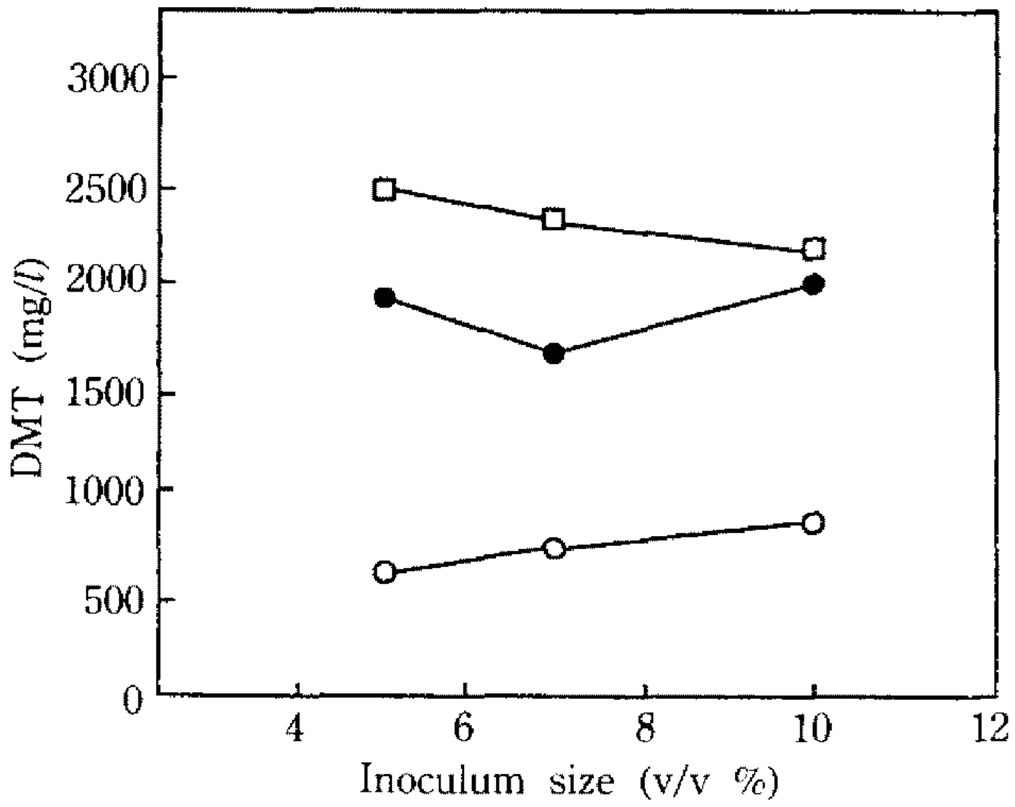

Fig. 4. Effect of inoculum size on DMT production.
○−○: 41 hr, ●−●: 89 hr, □−□: 161 hr

pH 6.7. pH of broth rose until age of 10 hours and then maintained between pH 6.5 and pH 6.7 naturally (Fig. 2). When pH of broth was controlled to be pH 6.8 by 20% ammonia water, the ammonium ion was accumulated and the rate of DMT productivity decreased sharply. Control of pH with sodium hydroxide showed the same results. Nevertheless, the concentration of ammonium ion did not increase. This phenomena implied that poor DMT productivity was not caused by the accumulation of ammonium ion. In the physiological point of view, it seems that the decrease of DMT production rate is caused by the pH level itself. These results must have come from the disturbance of microbial environment by alkali addition (Fig. 3).

Effect of inoculum size

Fermentation was carried out with different inoculum sizes ranging from 5% to 10%. Consumption rate of sugar was proportional to inoculum size in the early phase of fermentation, but reversed after the middle phase (Table 2). Pattern of DMT production was shown to be the same as that of sugar consumption (Fig. 4).

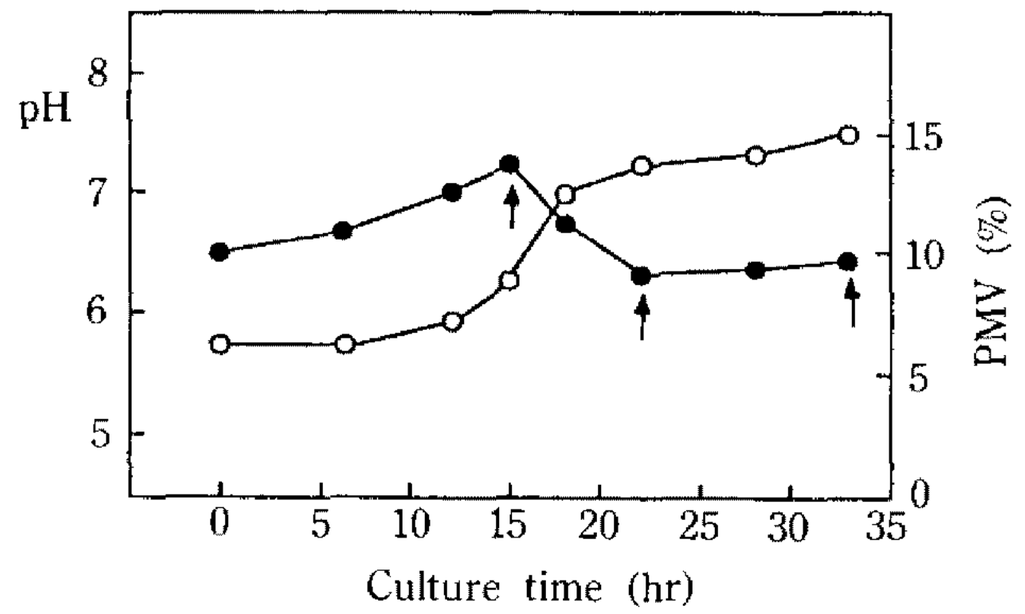

Fig. 5. A typical time course of pre-culture.
●−●: pH, ○−○: PMV

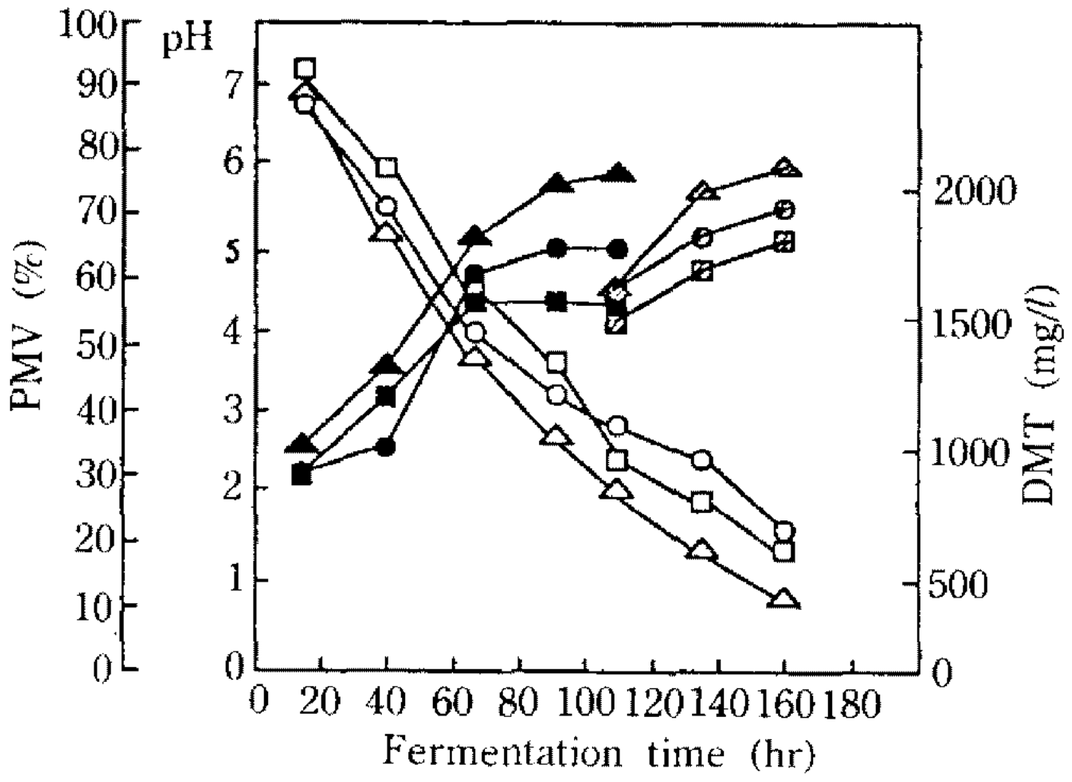

Fig. 6. Effect of phases on DMT fermentation.
○−○: 1st phase (15 hr) ○: Sugar, △−△: 2nd phase (22 hr) ●: PMV, □−□: 3rd phase (35 hr) ○: DMT

Effect of seed phase on DMT production

Fig. 5 shows a typical time clapse of seed culture of DMT producing strain. Cell mass did not change after 22 hours of cultivation, whereas, pH of broth varied as a function of time. So we transferred the seed culture to main medium. Each of arrows in Fig. 5 shows the transferring time of seed culture. The highest DMT productivity was obtained using the seed culture whose pH reached to a mimimum level. These indicated that pH of seed-culture was more important than cell mass (PMV) in determining the phase of seed. As mentioned before, DMT fermentation appeares to coincide with the general characters of fermentation of secondary metabolites, in which the proper rate of primary metabolism

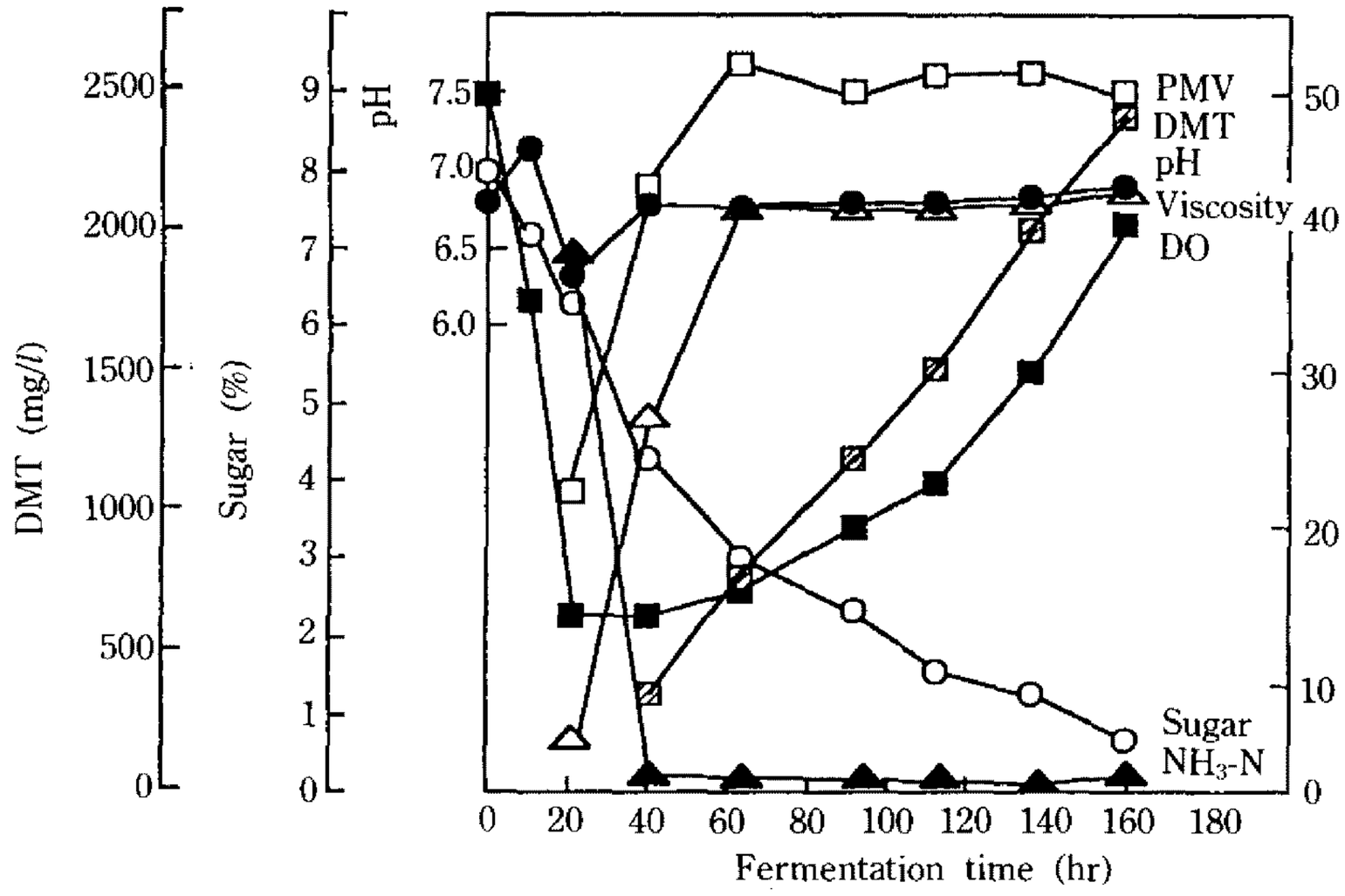

Fig. 7. A time course of DMT fermentation in pilot fermentation.
●-●: pH, ▲-▲: NH_3-N, ■-■: DO, ○-○: Sugar, △-△: Viscosity, □-□: PMV, □-□: DMT

is essential to massive production of secondary metabolites. Rate of primary metabolism can be estimated by analyzing the rate of sugar consumption, increase of PMV, and viscosity of broth and pH pattern. Quality of pre-culture must be considered. Considering the results obtained, fermentation was carried out in pilot scale. The final concentration of DMT has reached about 2200 mg/*l* and its content in whole derivatives was 76.5% (Fig. 7).

요 약

Demeclocycline 생산균주를 세포 융합하여 얻은 변이주를 이용하여 demethyltetracycline을 생산하기 위한 발효조건에 대해 연구한 결과, 발효 최적온도는 25℃였고, 초기 pH는 6.7였으며 발효기간 중 pH를 일정하게 조절하면 생산성이 저하되었다. 또한 식균량이 많을수록 초기 당 소모와 점도가 높았는데, 이는 식균량이 증가함에 따라 발효가 급격히 진행됨을 나타냈다. 식균량은 5% (v/v)일 때 생산성이 가장 높았으며 전배양 시간과 전배양 pH가 중요한 요인임을 밝혔다.

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