

CHAPTER III

RESULTS

1. Growth Cycle of *Streptococcus pyogenes* (Group A) C 203 S in Todd Hewitt Broth

As illustrated in Figure 5, the culture was in the exponential phase (log phase) for approximately five hours, from the seventh to the twelfth hour of the cultivation. In this phase, cells are very active and growth increases rapidly, especially at the mid-log phase which is the most active phase for cell division. In order to shorten the long incubation period, the eighth hour culture, which is near the mid-log phase, was chosen to be the starter (Chapter II, section 2.2) for the preparation of SLO throughout this study.

2. Optimization for High Streptolysin O Production

2.1 The Effect of the Incubation Period

As shown in Figures 6, 7 and 8, there was no SLO in cultural filtrate during 0 - 2 hr of incubation which was accompanied by a rapid increase in growth and a decrease in pH. After that, SLO increased rapidly and reached a maximum level at the fourth hour of the cultivation while growth reached the exponential phase (log phase) SLO level then rapidly decreased while growth and pH became constant.

Therefore, 4 hr was the optimum incubation period for SLO production by the group A *Streptococcus pyogenes* strain C 203 S in

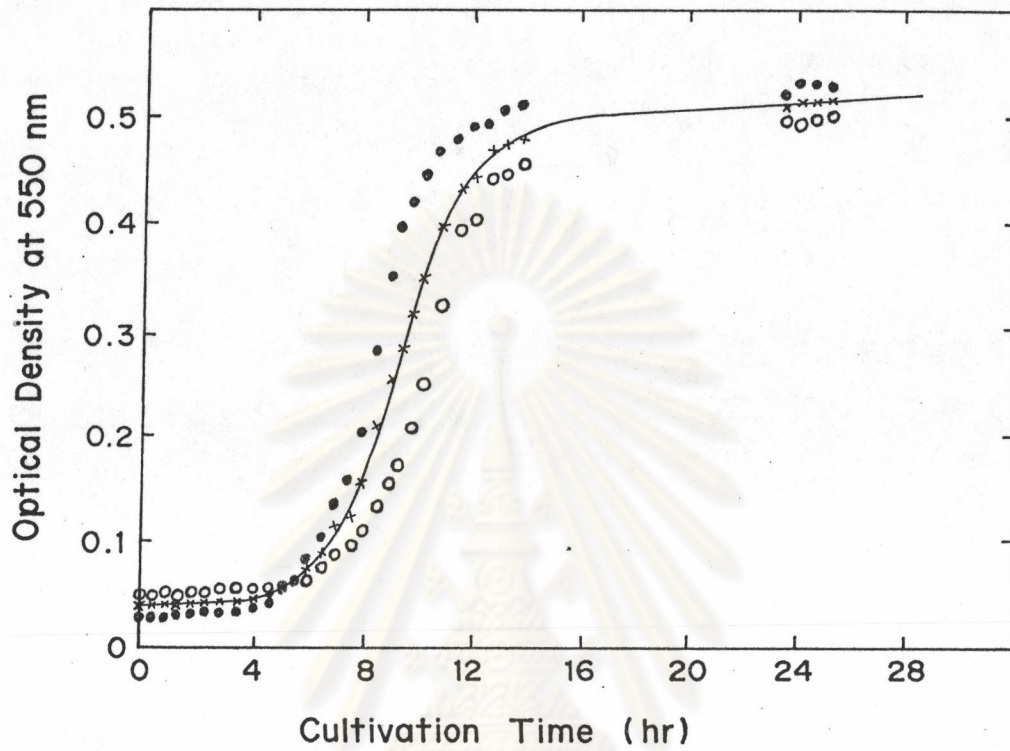


Figure 5 Growth Curve of Streptococcus pyogenes
(Group A) C 203 S in Todd Hewitt Broth
at 37°C

● and ○ OD_{550 nm} of Each Run
x—x Means of the OD_{550 nm} from
the Two Runs

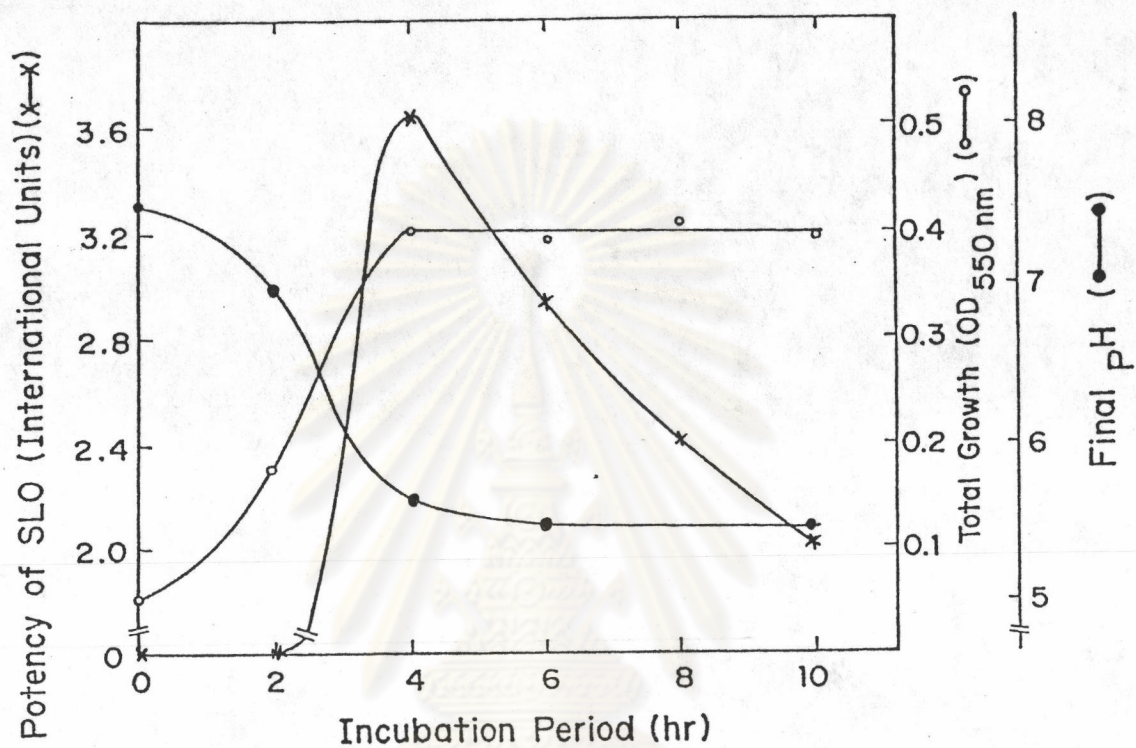


Figure 6 Potency of SLO, Total Growth and Final pH of the Culture at Various Incubation Periods (0 - 10 hr)

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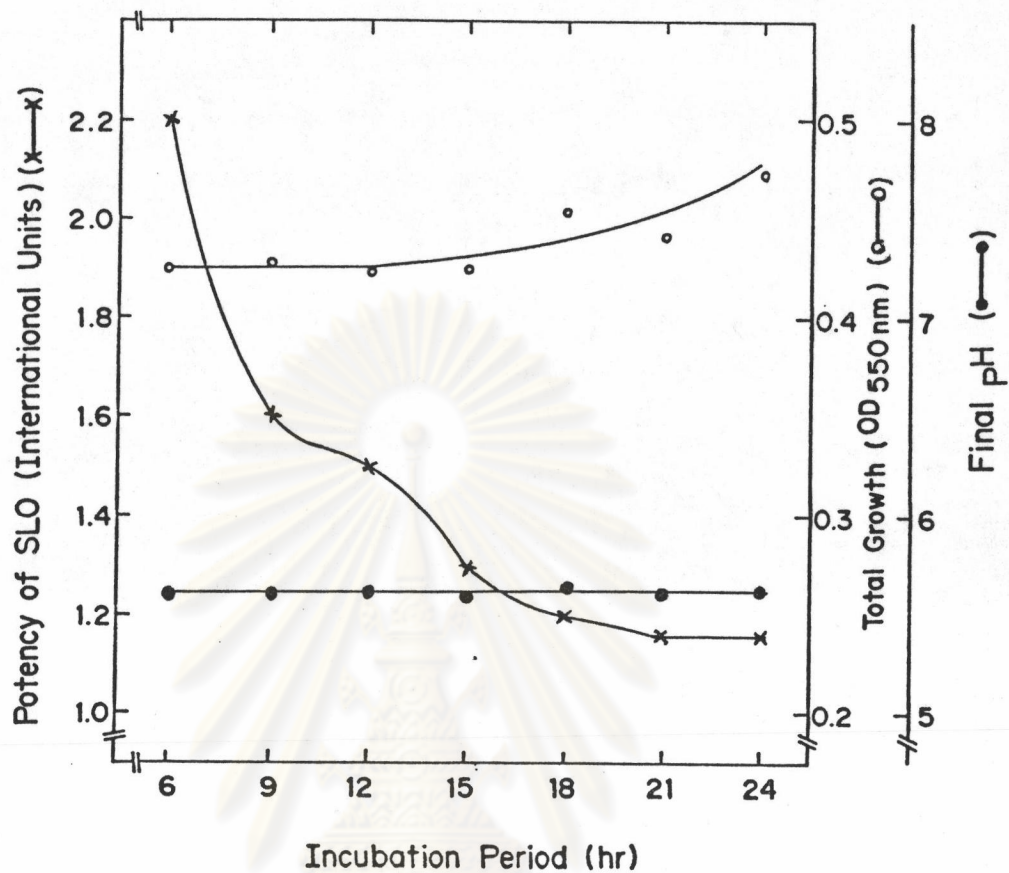


Figure 7 Potency of SLO, Total Growth and Final pH of the Culture at Various Incubation Periods (6 - 24 hr)

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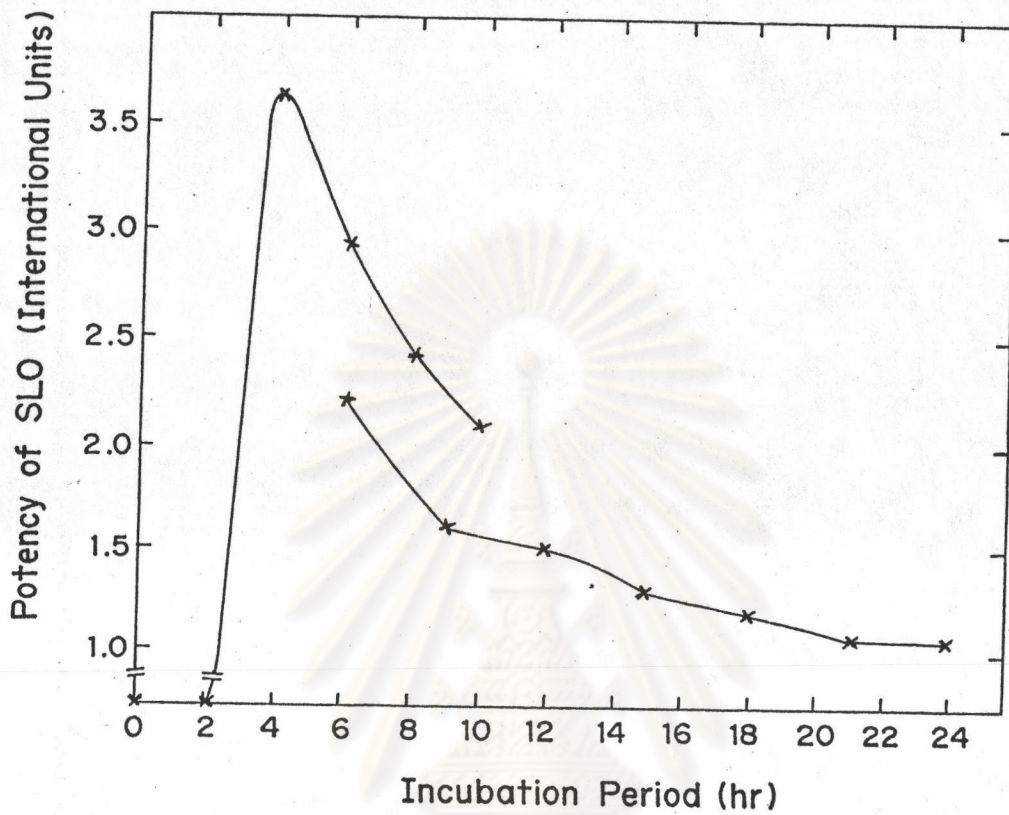


Figure 8 Potency of SLO from the Culture at Various Incubation Periods (0 - 24 hr)

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Todd Hewitt broth and this would be used in further experiments.

2.2 The Effect of the Age of Starter

As illustrated in Figure 9, starters (Chapter II, section 2.2) which were 10 - 12 hr of age yielded maximum growth in the step of SLO preparation (Chapter II, section 2.3). This was found concurrently with maximum SLO level and minimum pH of cultural fluid. Before and after this period, total growth was low and resulted in low level of SLO. Thus the optimum age of starter for SLO production by Streptococcus pyogenes (group A) C 203 S in Todd Hewitt broth was quite broad and in the range of 10 - 12 hr.

2.3 The Effect of the Size of Starter

When the size of starter was varied between 5 - 15 % of fresh medium it was found that total growth gradually increased while pH gradually decreased, as shown in Figure 10, and the highest level of SLO occurred when the starter's size was 15 % of fresh medium. Therefore, the appropriate size of starter for SLO production in Todd Hewitt broth by the strain C 203 S of Streptococcus pyogenes (group A) was approximately 15 % of fresh medium.

2.4 The Effect of Temperature

At 25°C, total growth was very low and there was no SLO detected, as shown in Table 7. Thus 37°C was much appropriate for SLO production by the strain C 203 S in Todd Hewitt broth.

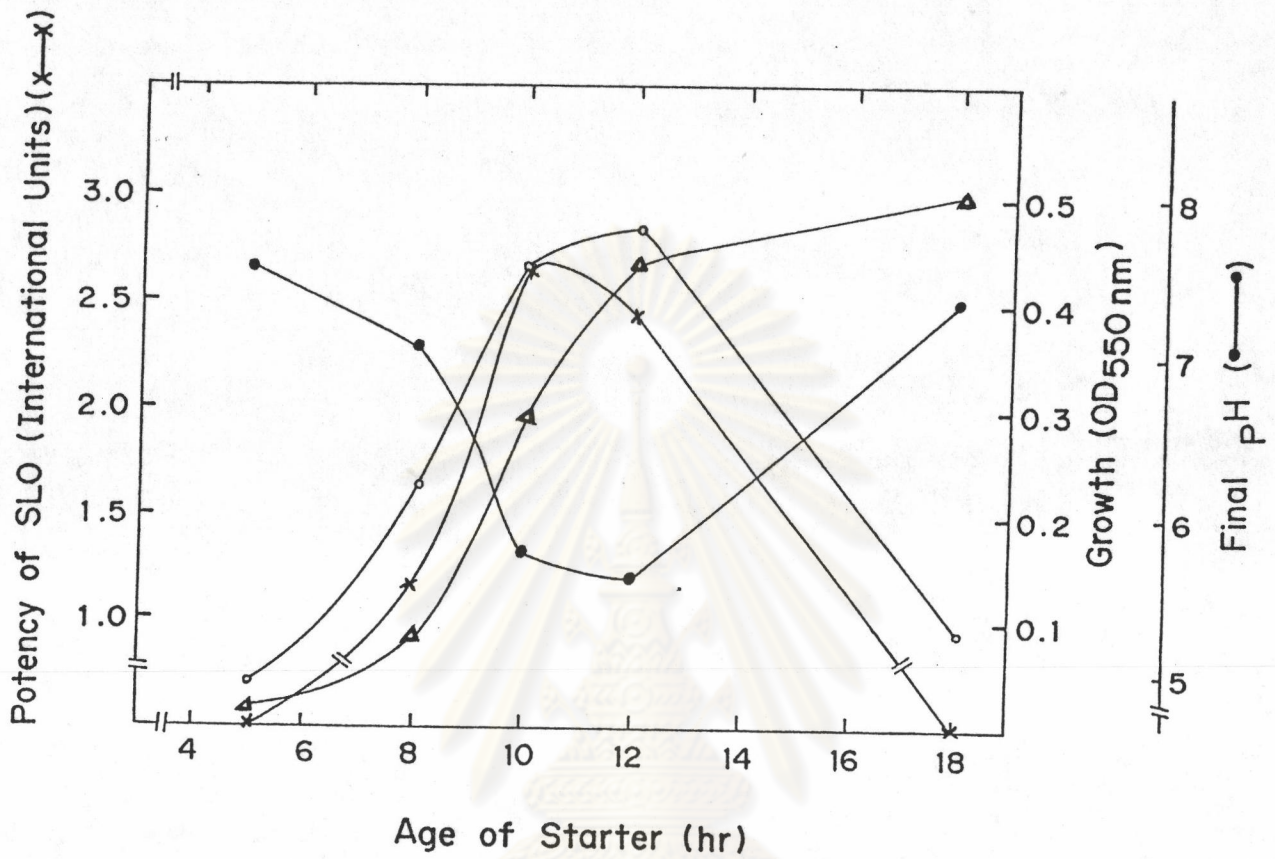


Figure 9 Potency of SLO, Growth of Starter (▲-▲) Total Growth (○-○) and Final pH of the Culture at Various Starter's Ages

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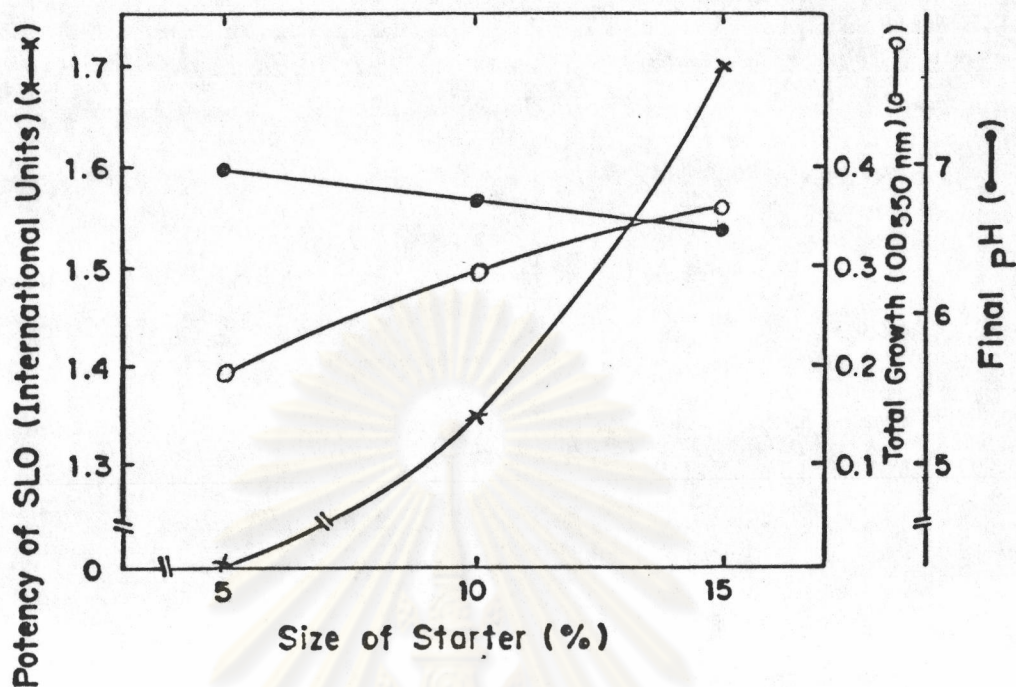


Figure 10 Potency of SLO, Total Growth and Final pH of the Culture at Various Starter's Sizes

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Table 7 Comparison of Potency of SLO, Total Growth and Final pH of the Culture at 25 and 37°C

Incubation Temperature (°C)	Potency of SLO (International Units)	Total Growth (OD _{550 nm})	Final pH
25	0	0.152	7.14
37	1.35	0.295	6.79

2.5 The Effect of the Initial pH

As shown in Figure 11, SLO could not be detected in cultural filtrate when initial pH of the cultivation was 6.6. It was then found to increase and reach a maximum level at initial pH of 7.8 concurrently with a maximum level in growth. After this point, both SLO level and growth were found to decrease. Therefore, the optimum initial pH for SLO production by the strain C 203 S in Todd Hewitt broth was approximate 7.8.

2.6 The Effect of Carbondioxide (CO₂) and Agitation

As illustrated in Table 8, Growth was much higher when the culture was incubated in atmospheric CO₂ than in 5 % CO₂ where there was no SLO detected. In atmospheric CO₂, SLO obtained from the standstill culture was a little higher than SLO obtained from the stirred culture.

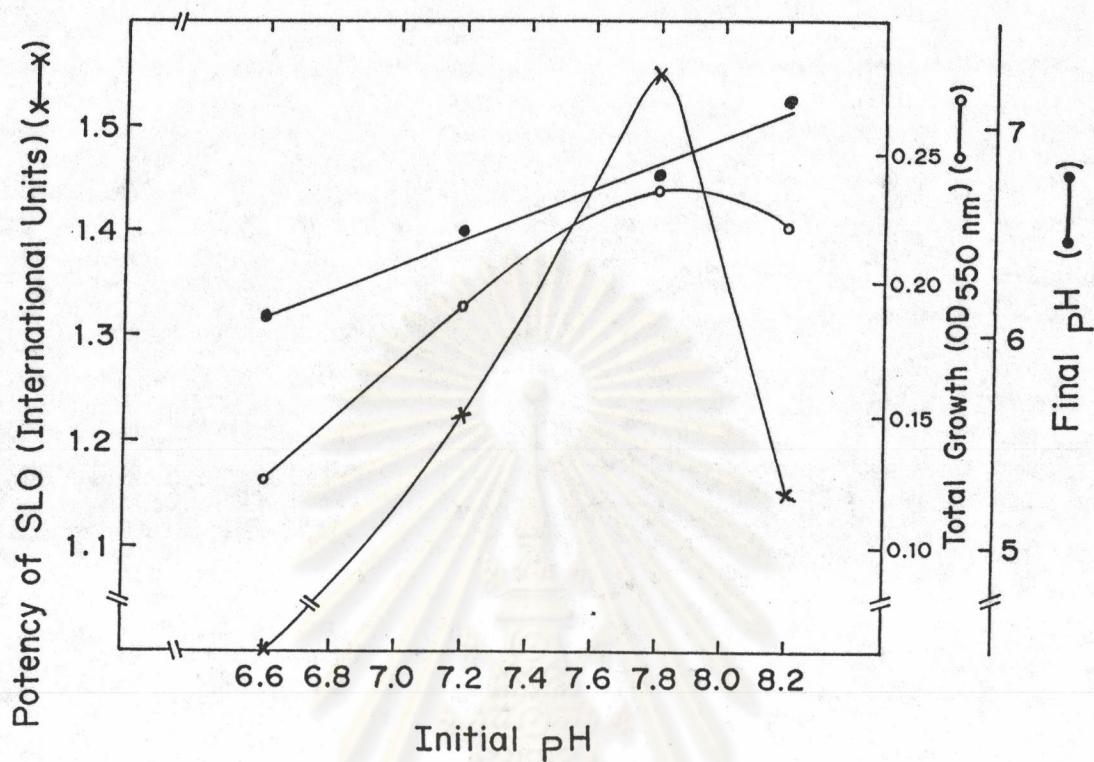


Figure 11 Potency of SLO, Total Growth and Final pH of the Culture Incubated at Various Initial pH

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Table 8 Effect of Carbondioxide and Agitation on Growth and SLO Production

CO ₂ Content	Agitation	Potency of SLO (International Units)	Total Growth (OD _{550 nm})	Final pH
Atmospheric CO ₂	Standstill	2.6	0.407	5.79
Atmospheric CO ₂	Stirred	2.4	0.404	6.03
5 % CO ₂	Standstill	0	0.224	6.57
5 % CO ₂	Stirred	0	0.220	6.52

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