

Chapter IV



RESULTS

4.1 Part I Cultivation of Candida utilis in shake flask apparatus.

The growth curves of C. utilis on pineapple juice substrate supplemented with individual or combinations of ingredients were studied. In Figure 2, the growth curves of C. utilis in media 8 and 9 are compared. The difference in growth is very clear from the early stage of growth. After 18 hours of cultivation, the yeast growth in the medium 9 remained rather constant at the absorbance about 2 until the end of the experiment while that in medium 8 was increasing to 11.64. It appeared that C. utilis grew in medium 8 more rapidly than medium 9. This indicated that ammonium sulfate was essential in the growth of C. utilis on the pineapple juice. Phosphorus and potassium were shown to be the principal mineral requirement of the yeast (Frey, et al., 1936; Nolte et al., 1942; Reiser, 1954). The effect of these nutrients is shown in Figure 3. Although the growth curves of C. utilis differ only a little, after the end of nine hours the growth in medium 1 is slightly better than that in medium 8. At the end of 64 hours cultivation, the absorbance reading of medium 1 was 16.32 compared with 11.64 from medium 8. It was very high when compared with the value of 2.25 obtained

Figure 2

The growth curves of C. utilis in media 8 and 9. The yeast was grown in the shake flasks at the speed of 240 rpm.

Cultivation condition : pH-4.0, temperature - 30°C,
cultivation times - 64 hours

The medium composition was,

medium 8 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v)

medium 9 - invert sugar 2% (w/v)

● medium 8

○ medium 9

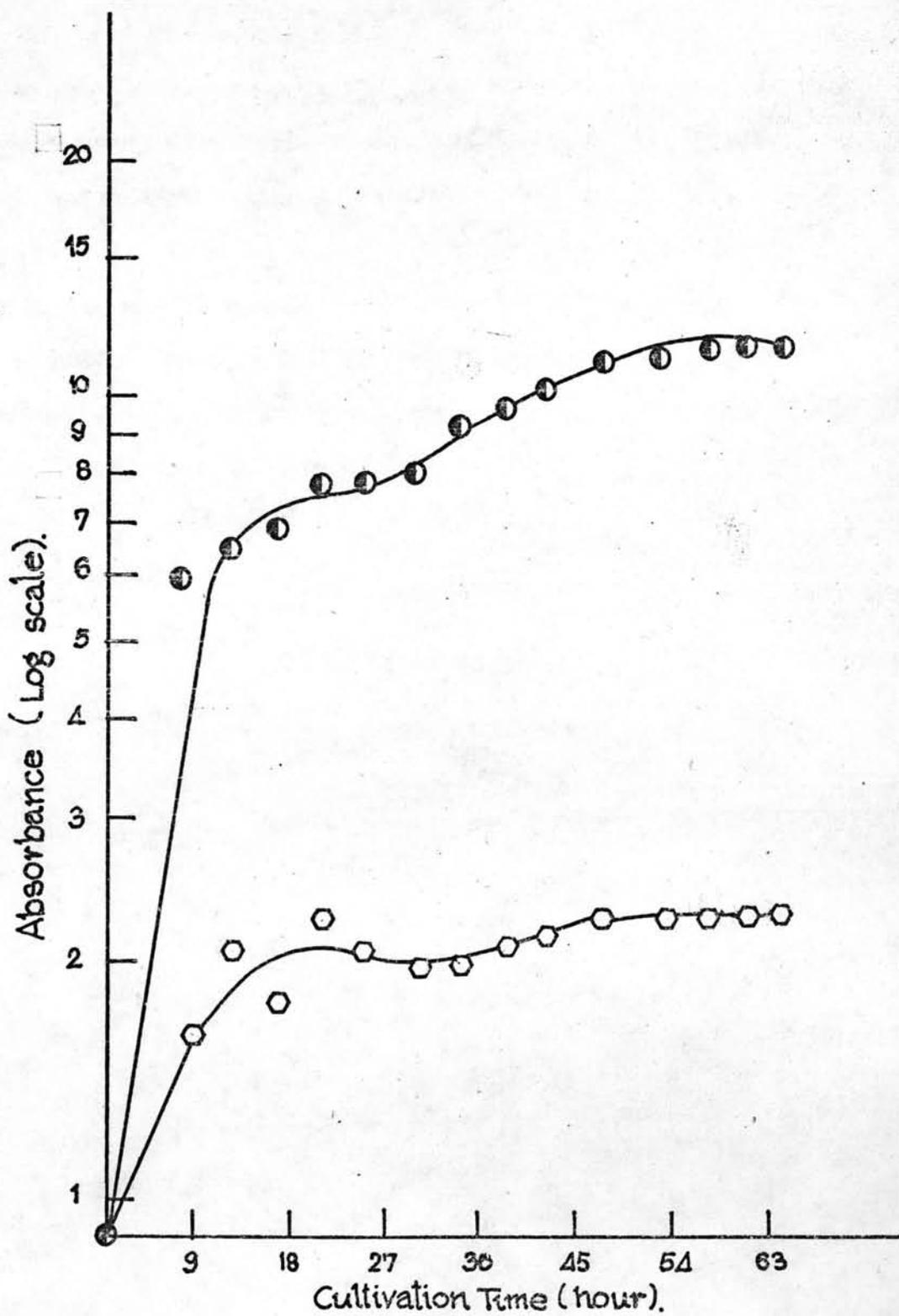


Figure 3

The growth curves of C. utilis in media 1 and 8. The yeast was grown in the shake flasks at the speed of 240 rpm.

Cultivation condition : see Figure 2

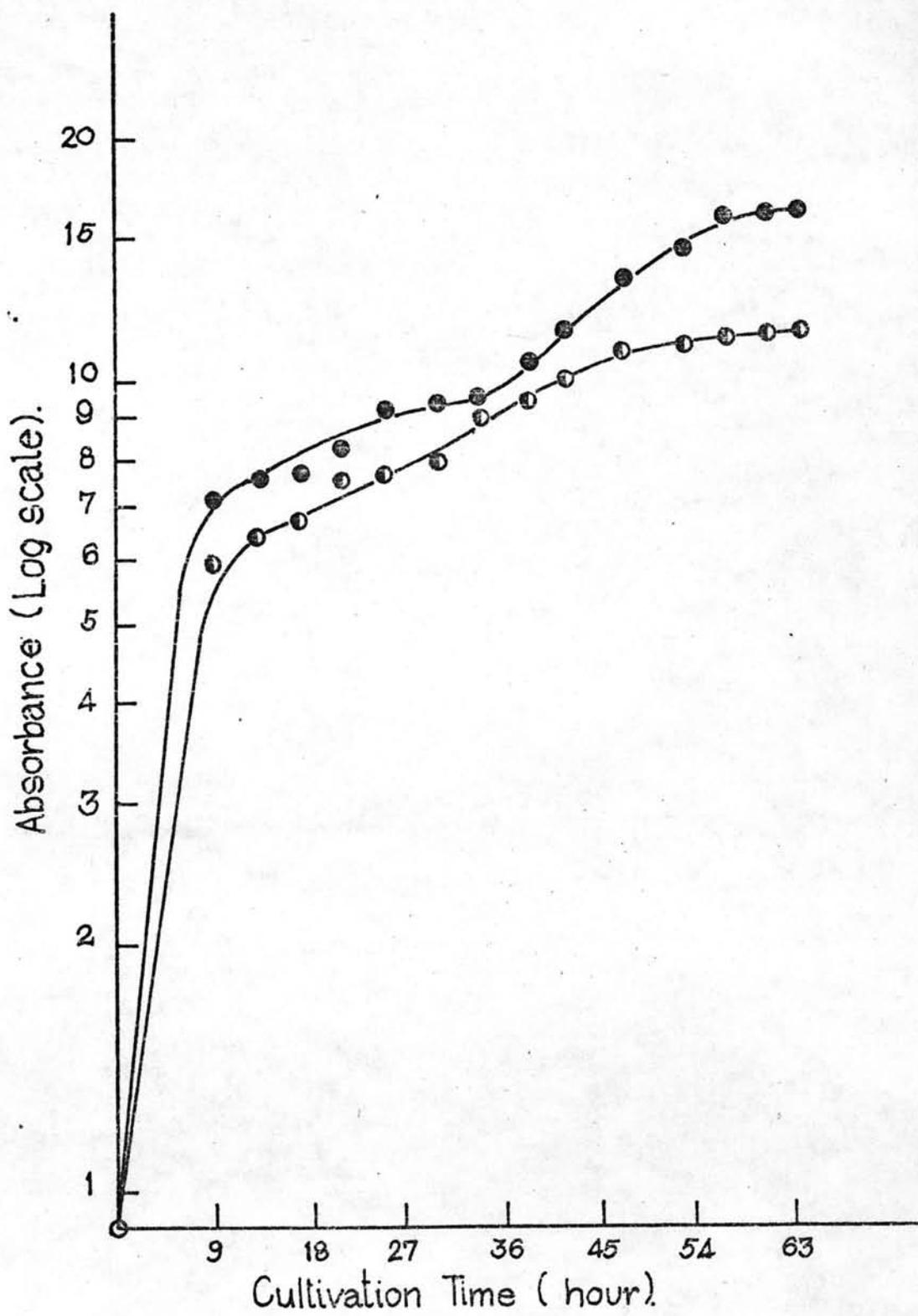
The medium composition was,

medium 1 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), KH_2PO_4
0.05% (w/v)

medium 8 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v)

● medium 1

○ medium 8



from the medium 9. So the supplement of potassium and phosphorus are required for better growth of C. utilis on pineapple juice. Yeast extract was added in medium 3 to study the requirement of vitamin B and unknown growth factors for the growth of C. utilis on the substrate (Figure 4). The growth of C. utilis in both media is not much different. The highest absorbance reading was about 16 in both media at the end of 64 hours cultivation. The values of the absorbance agrees well with the dry weight. In both media, the yeast dry weight was 8.23 g/l (Table 3). However, the yield of yeast obtained from medium 1 was slightly higher than that of medium 3 (see Table 3). The difference of the yields came from the variation in sugar consumption of both media. At the end of 64 hours cultivation, the sugar consumption in medium 3 was 97% compared with 95% in medium 1. This caused the yield of medium 3 lower than that of medium 1. Hence, it appeared that the production of yeast cell mass in the pineapple juice supplemented with $(\text{NH}_4)_2\text{SO}_4$, KH_2PO_4 at the cultivation condition employed did not require yeast-extract for good growth. The effect of supplementation inorganic or organic nitrogen sources for the growth of yeast was further studied. The addition of ammonium sulfate, peptone, and urea in media 1, 2, and 4 showed the effect of these different nitrogen sources (Figure 5). The yeast growth in medium 1 gave the highest absorbance reading at the end of 64 hours cultivation, followed by media 4 and 2 at the values of 16.32, 13.20, and 11.07, respec-

Figure 4

The growth curves of C. utilis in media 1 and 3. The yeast was grown in the shake flasks at the speed of 240 rpm.

Cultivation condition : see Figure 2

The medium composition was,

medium 1 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), KH_2PO_4 0.5% (w/v)

medium 3 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), KH_2PO_4 0.5% (w/v), yeast extract 0.1% (w/v)

● medium 1

△ medium 3

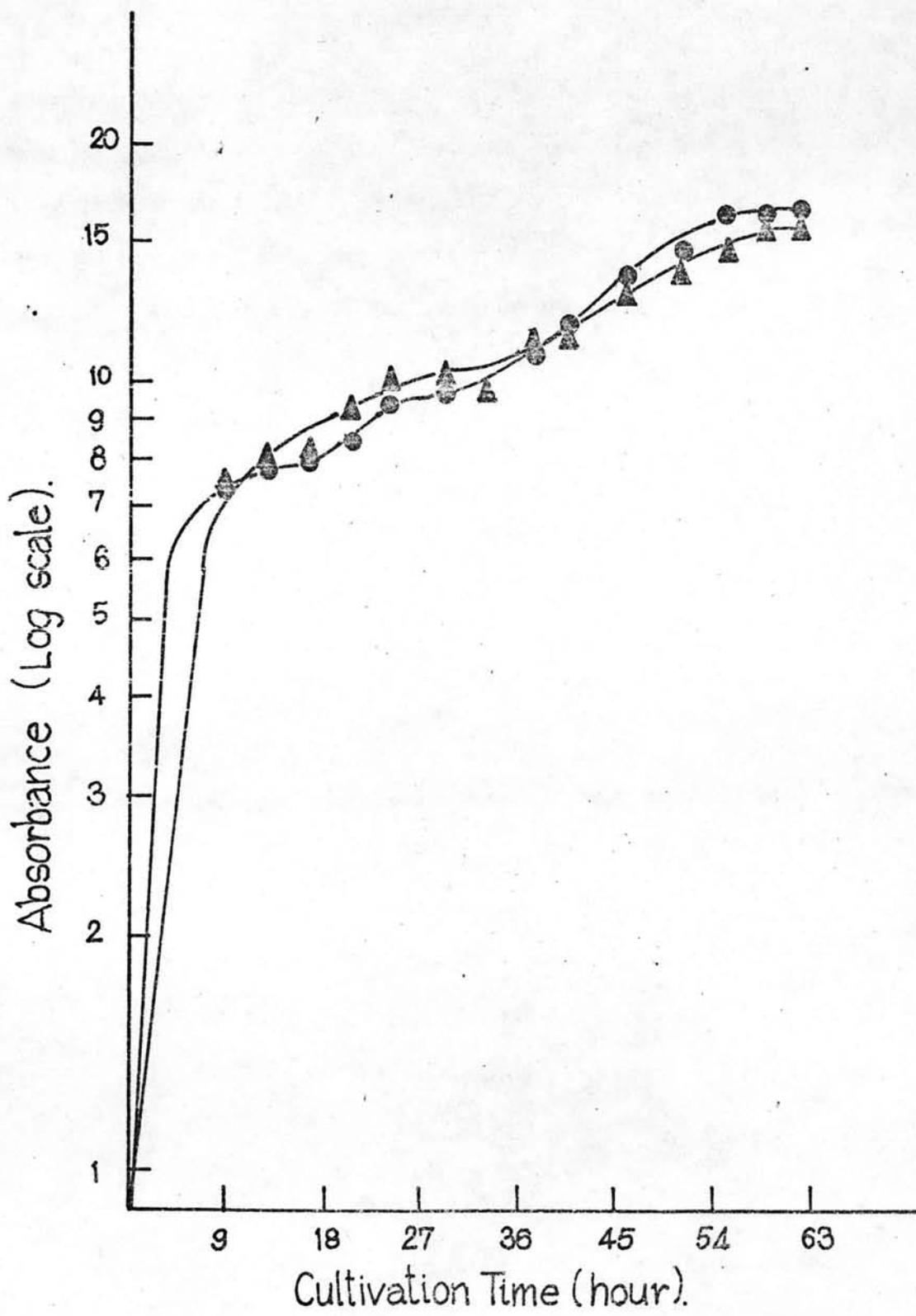


Figure 5

The growth curves of C. utilis in media 1, 2 and 4. The yeast was grown in the shake flasks at the speed of 240 rpm.

Cultivation condition : see Figure 2

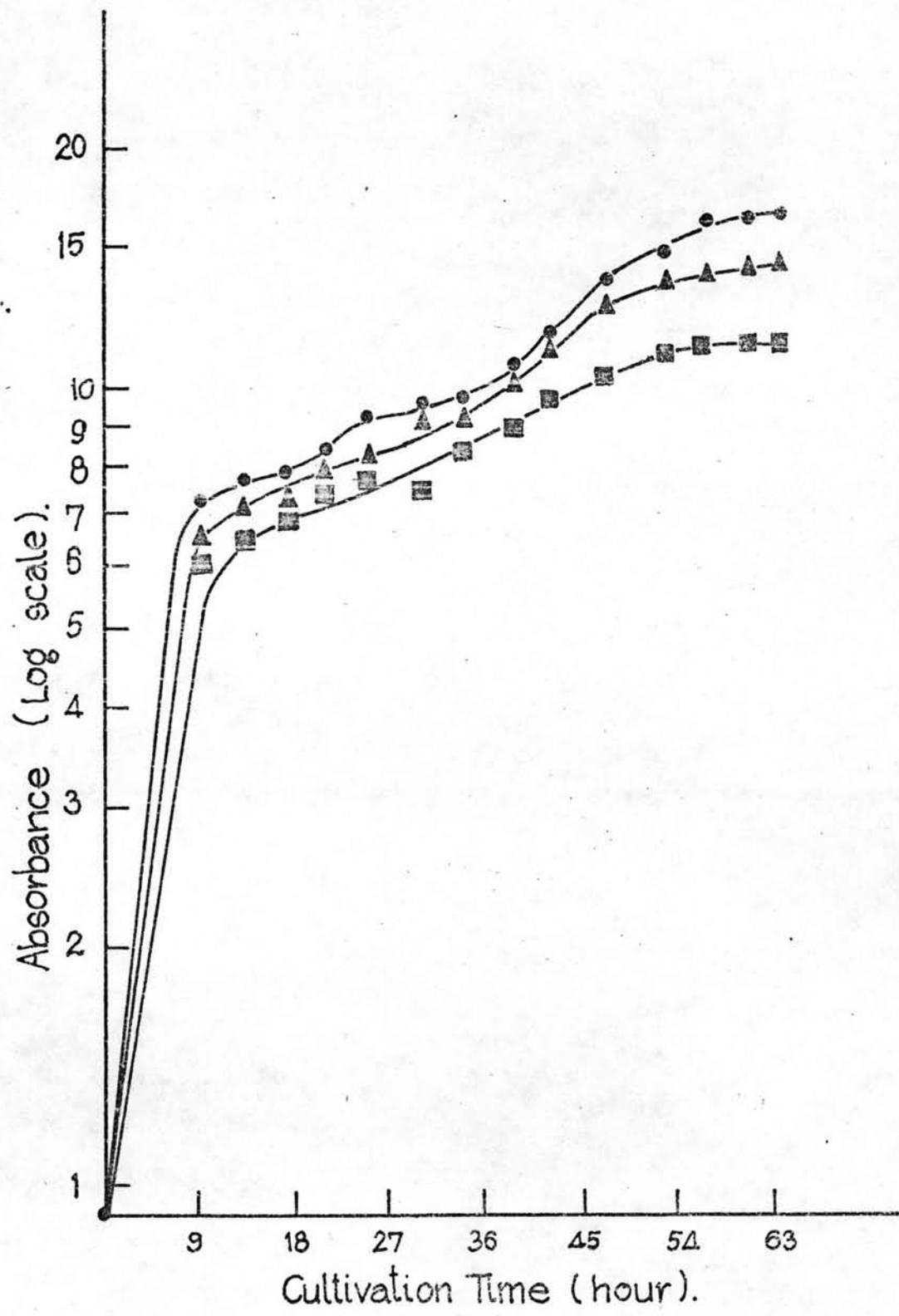
The medium composition was,

medium 1 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), KH_2PO_4 0.5% (w/v)

medium 2 - invert sugar 2% (w/v), peptone 0.5% (w/v), KH_2PO_4 0.5% (w/v)

medium 4 - invert sugar 2% (w/v), urea 0.03% (w/v), KH_2PO_4 0.5% (w/v)

- medium 1
- medium 2
- ▲ medium 4



tively. The yeast dry weight and yeast yield obtained from medium 4 was 7.76 g/l and 39.10% respectively (see Table 3). The yield of yeast obtained from medium 4 is a little lower than that from medium 1, i.e., 42.1%. Although the yield of yeast in medium 2 was not determined, it was rather clear from the growth curve that medium 2 gave the poorest result. From the data obtained in Figures 2-5, it indicated that pineapple juice contained insufficient nitrogen and phosphates for the growth requirement of the yeast, and it was, therefore, necessary to add these nutrients in the form of $(\text{NH}_4)_2\text{SO}_4$ and KH_2PO_4 to supply the deficiencies. Yeast extract was also added in medium 6 to provide the necessary growth factors for the assimilation of peptone (Figure 6). It appeared that the growth of yeast in medium 6 is slightly better than that from medium 2. This might be due to the presence of yeast extract. Nevertheless, the growth of yeast in medium 1 appeared to be superior. The absorbance readings at the end of 64 hours cultivation in the media 1, 2, and 6 were 16.32, 13.31 and 11.07, respectively. In contrast, when yeast extract was added in the medium containing ammonium sulfate and urea as nitrogen sources it showed no beneficial effect (Figure 7). The yeast growth in medium 4 is better than in medium 5 especially near the end of the cultivation (after 42 hours). The absorbance reading in medium 5 was 12.6 compared to 13.2 obtained from medium 4 after 64 hours cultivation. The growth curves of yeast in media 1

Figure 6

The growth curves of *C. utilis* in media 1, 2, and 6. The yeast was grown in the shake flasks at the speed of 240 rpm.

Cultivation condition : see Figure 2

The medium composition was,

medium 1 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), KH_2PO_4 0.5% (w/v)

medium 2 - invert sugar 2% (w/v), peptone 0.5% (w/v), KH_2PO_4 0.5% (w/v)

medium 6 - invert sugar 2% (w/v), peptone 0.5% (w/v), KH_2PO_4 0.5% (w/v), yeast extract 0.1% (w/v)

- medium 1
- medium 2
- medium 6

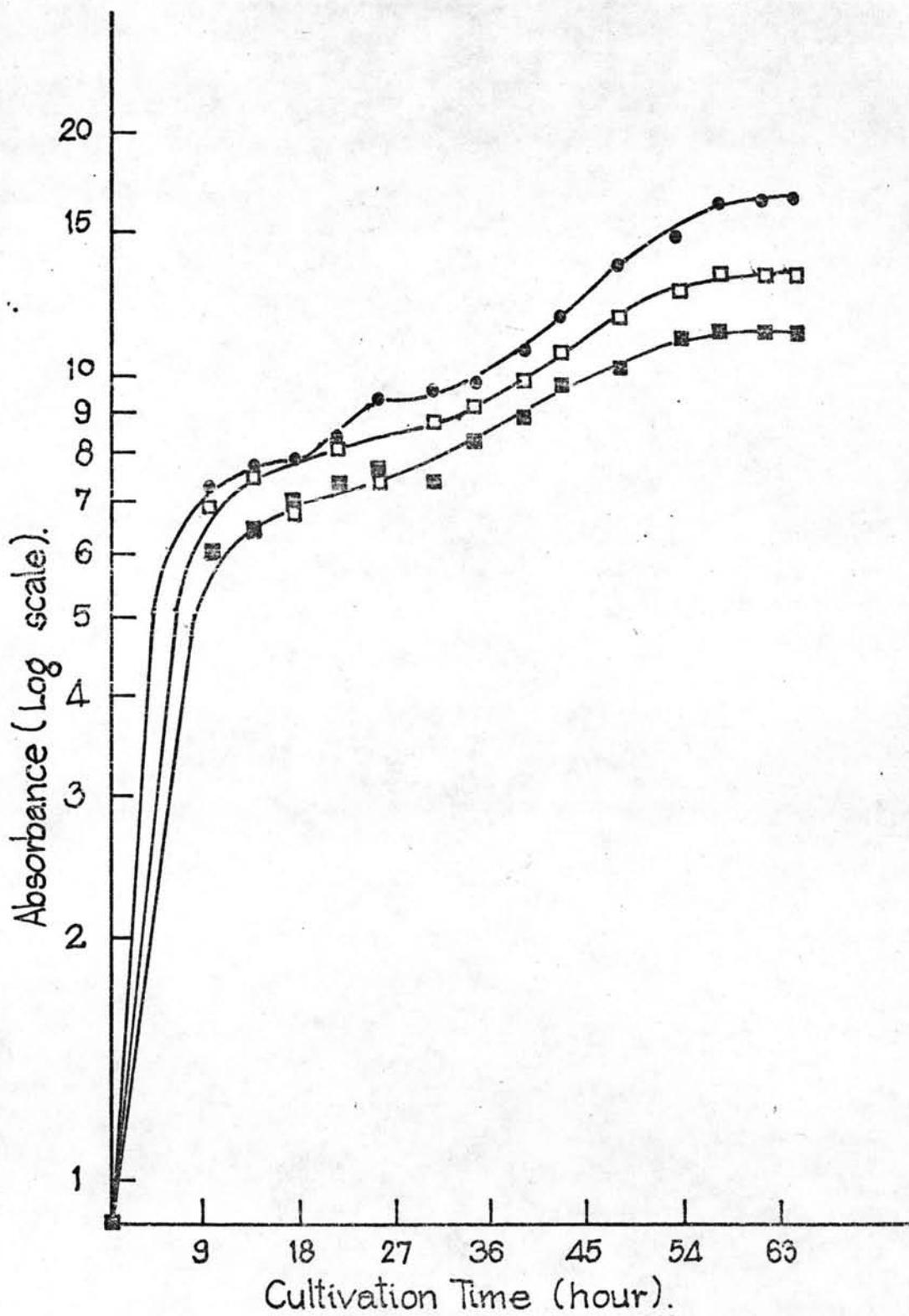


Figure 7

The growth curves of C. utilis in media 1, 4, and 5. The yeast was grown in the shake flasks at the speed of 240 rpm.

Cultivation condition : see Figure 2

The medium composition was,

medium 1 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), KH_2PO_4 0.5% (w/v)

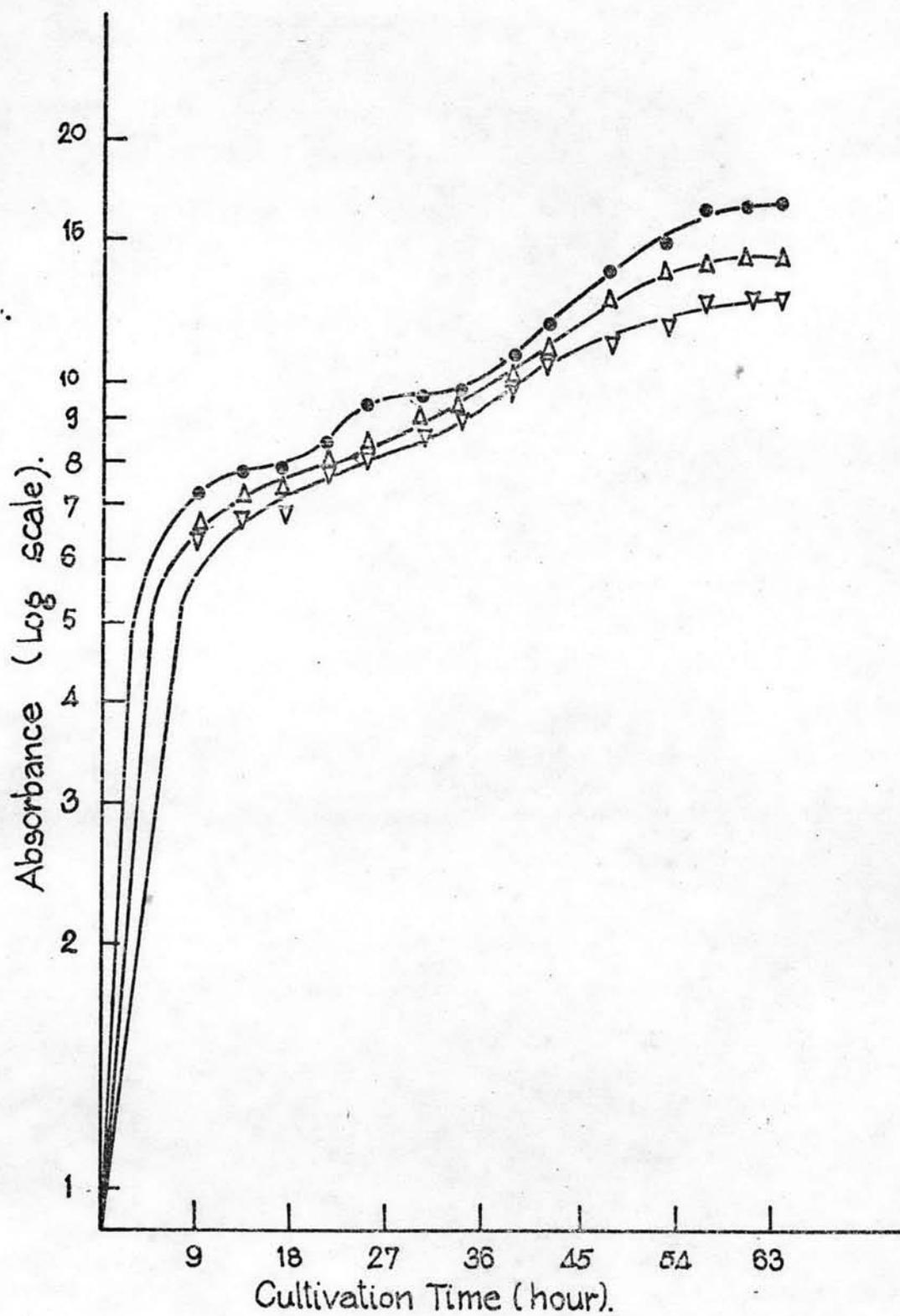
medium 4 - invert sugar 2% (w/v), urea 0.03% (w/v), KH_2PO_4 0.5% (w/v)

medium 5 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), urea 0.03% (w/v), KH_2PO_4 0.5% (w/v)

● medium 1

△ medium 4

▽ medium 5



and 7 are shown in Figure 8. Although medium 7 was supplemented with urea, peptone, and yeast extract in addition to ammonium sulfate and potassium dihydrogen phosphate. These nutrients were shown to fulfil the requirement of the growth of yeast (Prescott and Dunn, 1959; Pyke, 1958; Suomalainen and Oura, 1971). However, it appeared that the growth of yeast in medium 1 was slightly superior than medium 7 over the entire period of study. The absorbance reading and dry weight obtained from medium 7 were 12.79 and 6.80 g/l, respectively (see Table 3). According to the dry weight and sugar consumption, the yeast yield from media 1 and 7 were 42.1% and 38.2%. The consumption of sugar during the cultivation of yeast in media 1, 3, 4, and 7 is shown in Figure 9. The percentage of sugar consumptions were relatively high, exceeding 50% after 24 hours of cultivation and increased rapidly until the end of 57 hours. After that the rate of sugar consumptions leveled off as the cultivation proceeded. The amount of sugar consumed was about 95% for all media used except medium 7. Comparison of yield and protein content of C. utilis grown in media 1, 3, 4 and 7 are also tabulated (see Table 3). Yeast grown in medium 1 gave the highest yield as well as the protein content. The protein content of the yeast from medium 1 was found to be 55.2%. Hence, the medium 1 was selected among the nine media tried for further studied in the cultivation of C. utilis in stirred-vessel fermenter.

Figure 8

The growth curves of C. utilis in media 1 and 7. The yeast was grown in the shake flasks at the speed of 240 rpm.

Cultivation condition : see Figure 2

The medium composition was,

medium 1 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), KH_2PO_4 0.5% (w/v)

medium 7 - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v), urea 0.03% (w/v), peptone 0.5% (w/v), KH_2PO_4 0.5% (w/v), yeast extract 0.1% (w/v)

● medium 1

○ medium 7

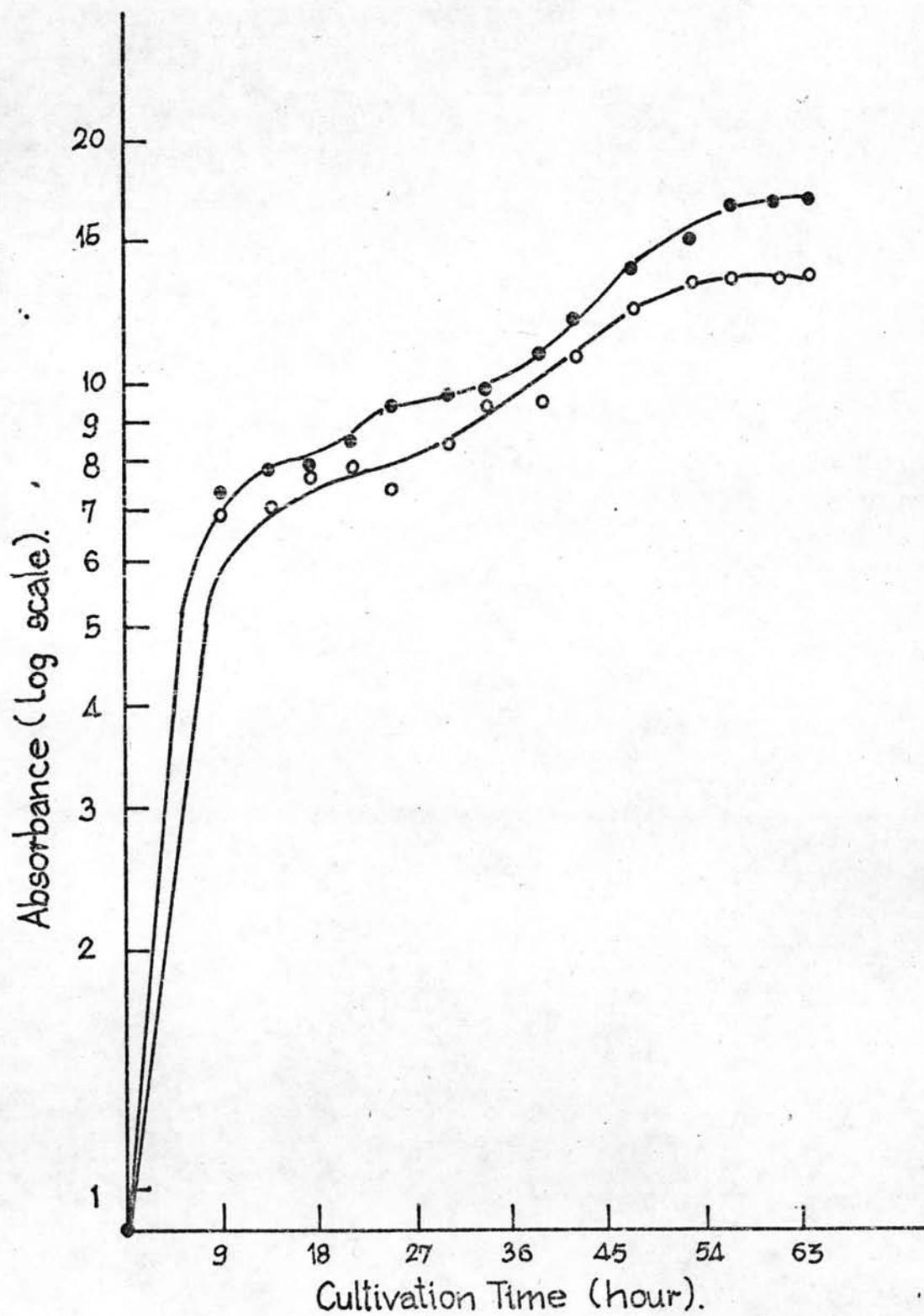


Figure 9

The consumption of sugar in media 1, 3, 4, and 7 during the growth of C. utilis in the shake flasks.

- medium 1
- △ medium 3
- medium 4
- medium 7

Cultivation condition : see Figure 2

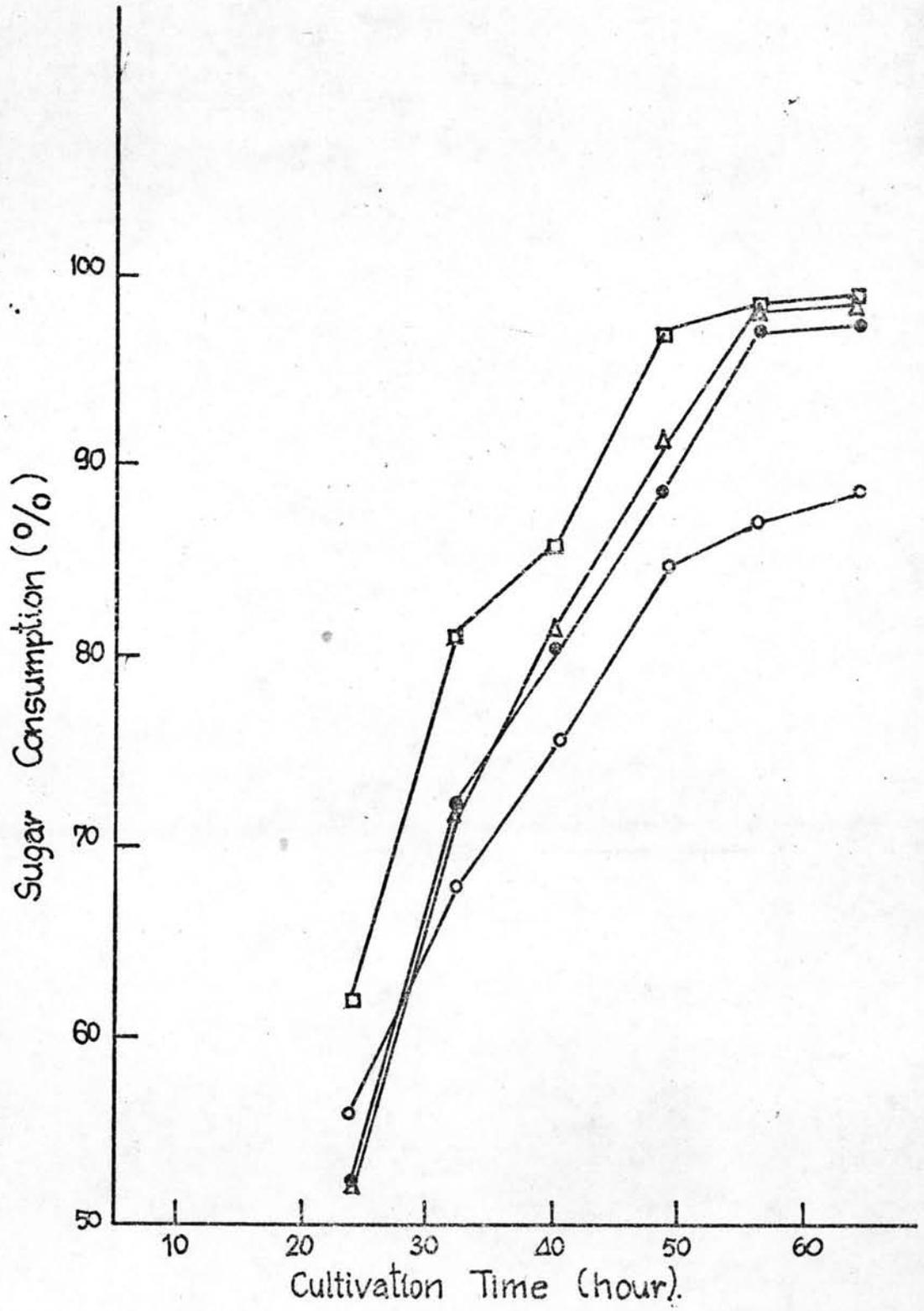


Table 3 Comparison of yield and protein content of Candida utilis grown in various media during shake flask experiments

	medium 1	medium 3	medium 4	medium 7
Dry weight (g/l)	8.22, 8.25, 8.22	8.22, 8.24, 8.23	7.74, 7.77, 7.74	6.75, 6.65, 6.80
average	8.23	8.23	7.76	6.80
Protein content (N x 6.25)	55.21, 55.19, 55.2	54.1, 54.1, 54.1	53.27, 53.30, 53.30	53.31, 53.34, 53.31
average	55.2	54.1	53.30	53.32
Yield (%)	42.05, 42.10, 42.15	41.8, 41.6, 41.7	39.18, 39.06, 39.06	38.22, 38.1, 38.19
average	42.10	41.7	39.10	38.2

4.2 Part II Cultivation of Candida utilis in stirred-vessel fermenter.

According to the results in Part I, the medium 1 was selected for the growth of C. utilis on pineapple juice. This part was undertaken to determine yeast dry weight, protein content, sugar consumption, and chemical oxygen demand as a function of agitation and aeration when C. utilis was grown in the medium 1. Because of the limitation of the designed apparatus, only three aeration rates were studied, i.e., 0.5, 1, and 1.5 volume of air per volume of culture medium per minute (VVM). Before starting the experiment, absorbance of the culture medium after inoculated with the yeast inoculum was adjusted at 1. Medium 1 was added to dilute the seed yeast when required. In Figure 10, the growth curves of C. utilis in medium 1 are shown at three aeration rates also with the yeast growth in the shake flask for comparison. The yeast grown in the fermenter was superior than in shake flask over the entire period of cultivation. Considering the yeast growth at the three aeration rates, it appeared that the best aeration rates was 1 VVM and the poorest one was 0.5 VVM. However, the growth curves of C. utilis at three aeration rates were similar. During the first five hours of cultivation, the yeast growth was increased considerably, then it appeared unchanged until the end of 8 hours. In the media aerated at 1 and 1.5 VVM, the yeast growths were quite

Figure 10

The growth curves of C. utilis in medium 1. The yeast was grown in stirred vessel fermenter at aeration rates 0.5, 1, 1.5 VVM.

Cultivation condition : agitation speed - 1400 rpm.,

pH - 4.0, temperature - 30°C

medium composition - invert sugar 2% (w/v), $(\text{NH}_4)_2\text{SO}_4$ 0.5% (w/v)

KH_2PO_4 0.5% (w/v)

■ 0.5 VVM.

● 1 VVM.

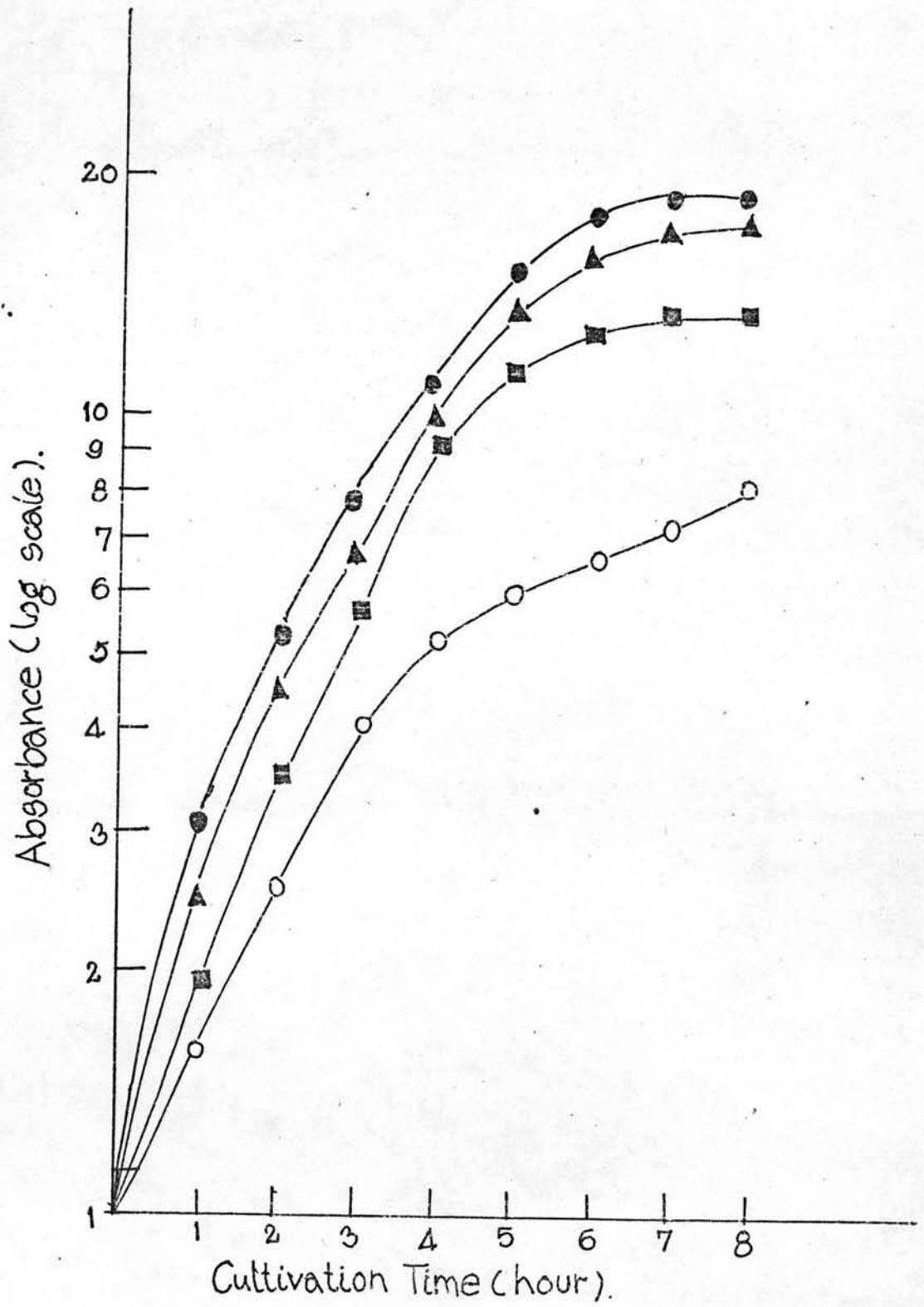
▲ 1.5 VVM.

○ in shake flask apparatus,

cultivation condition :

pH - 4.0, temperature - 30°C

vibrating speed 240 rpm.



close over the entire period of the cultivation. The absorbance readings after 8 hours were 18.01, 17.70 and 13.67 at the aeration rates of 0.5, 1, and 1.5 VVM, respectively, compared with 7.87 from the shake flask study. Absorbance reading and the dry weight obtained agreed well i.e., the highest absorbance gave the highest yeast dry weight (Table 4). The yeast dry weight and yeast yield were 9.5 g/l and 49.41% when the yeast was grown in the fermenter at the end of 8 hours, whereas the corresponding values of 8.23 g/l and 42.1% were obtained when the yeast was grown in the same medium but with the shake flask apparatus at the end of 64 hours. However, the protein content of the yeast cultivated from the same medium was independent of the aeration rates studied. The average protein content obtained from yeast grown in medium 1 was 55.38%. The percentage of sugar consumed was in the same magnitude as in the shake flask experiment (see Figure 9 and 11). All of the sugar consumption curves seemed to divide into two phases. In the first phase, the rate of sugar consumption was high whereas the second phase, the rate was fairly constant. The sugar consumption was almost 100% for three aeration rates used in the fermenter. The rate of sugar consumption in fermenter was higher than that in the shake flask. This might be due to the inadequate amount of air supplied to the yeast grown in the shake flask. Relationship of yeast dry weight and cultivation time is shown in Figure 12. The yeast dry weight was almost linear relationship with culti-

Table 4 Comparison of yield and protein content of Candida utilis grown in medium 1 during batch cultivation at different aeration rates

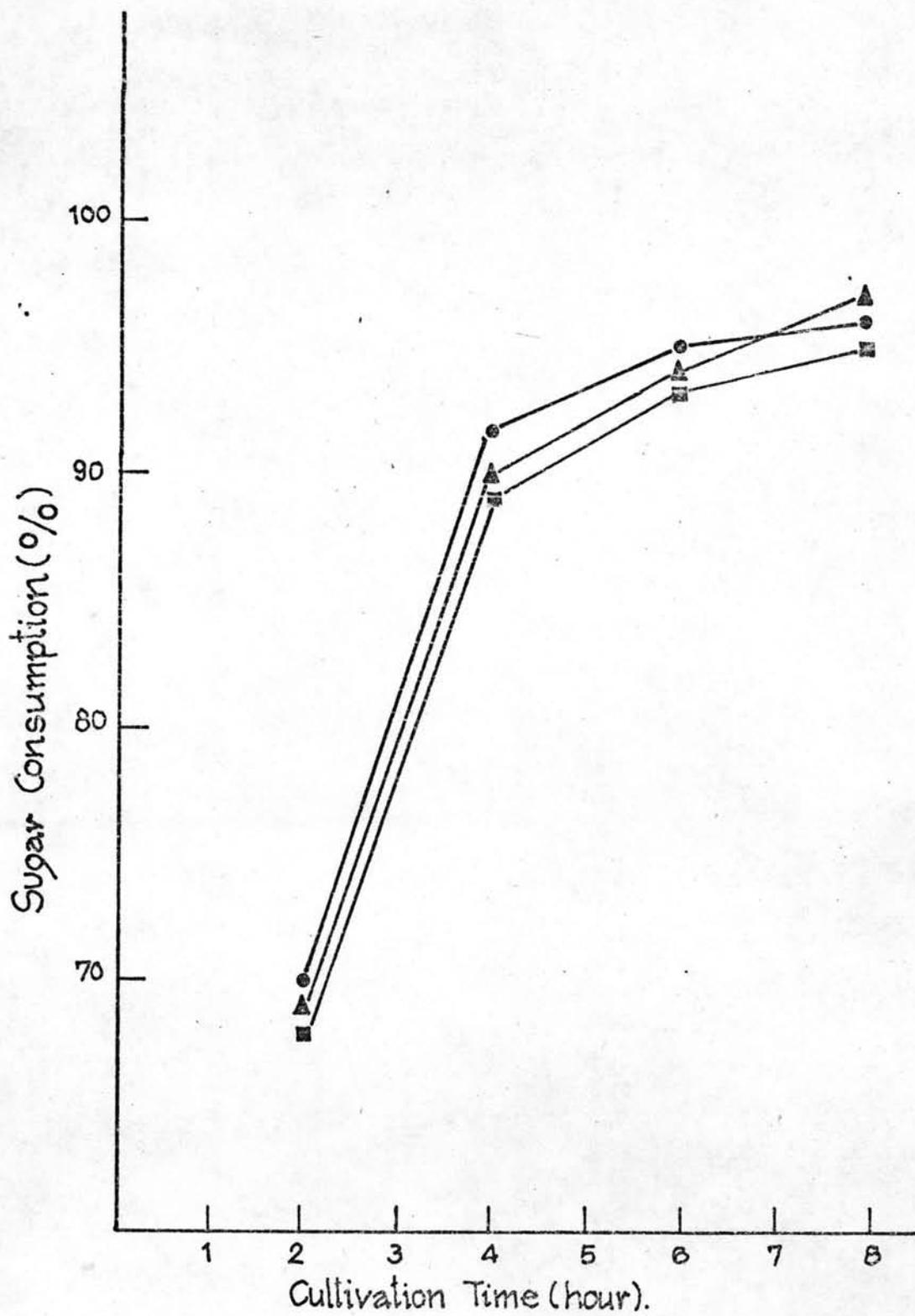
	flow rate of air		
	0.5 VVM	1 VVM	1.5 VVM
Dry weight (g/l)	8.63, 8.77, 8.70	9.54, 9.48, 9.48	8.94, 8.98, 8.97
average	8.70	9.50	8.96
Protein content (N x 6.25)	55.11, 55.13, 55.12	55.41, 55.48, 55.49	55.34, 55.39, 55.38
average	55.12	55.46	55.37
Yield (%)	45.72, 45.76, 45.79	49.39, 49.42, 49.42	46.15, 46.23, 46.22
average	45.76	49.41	46.20

Figure 11

The consumption of sugar in medium 1 during the growth of C. utilis in stirred vessel fermenter at aeration rate 0.5, 1, 1.5 VVM.

Cultivation condition : see Figure 10

- 0.5 VVM.
- 1 VVM.
- ▲ 1.5 VVM.



vation time for the three aeration rates studied. In general, the sugar consumption results in COD reduction. The reduction of COD increased with cultivation time in the same manner as the dry weight (Figure 12 and 13). At the end of 8 hour cultivation with 1 and 1.5 VVM aeration rates the reduction of COD was about 78% while that with 0.5 VVM it was about 70%. The relationship of sugar consumption and COD reduction is shown in Figure 14. The reduction of COD appeared to increase with the consumption of sugar at all aeration rates studied.

In conclusion, C. utilis grown on pineapple juice at 2% invert sugar supplemented with 0.5% (w/v) ammonium sulfate and 0.5% (w/v) potassium dihydrogen phosphate gave better growth when cultivated in stirred-vessel fermenter at agitation speed of 1400 rpm. and aeration rate of 1 VVM.

Figure 12

Comparison of dry weight of C. utilis grown in medium 1
at various aeration rates in stirred vessel fermenter.

Cultivation condition : see Figure 10

■ 0.5 VVM.

● 1 VVM.

▲ 1.5 VVM.

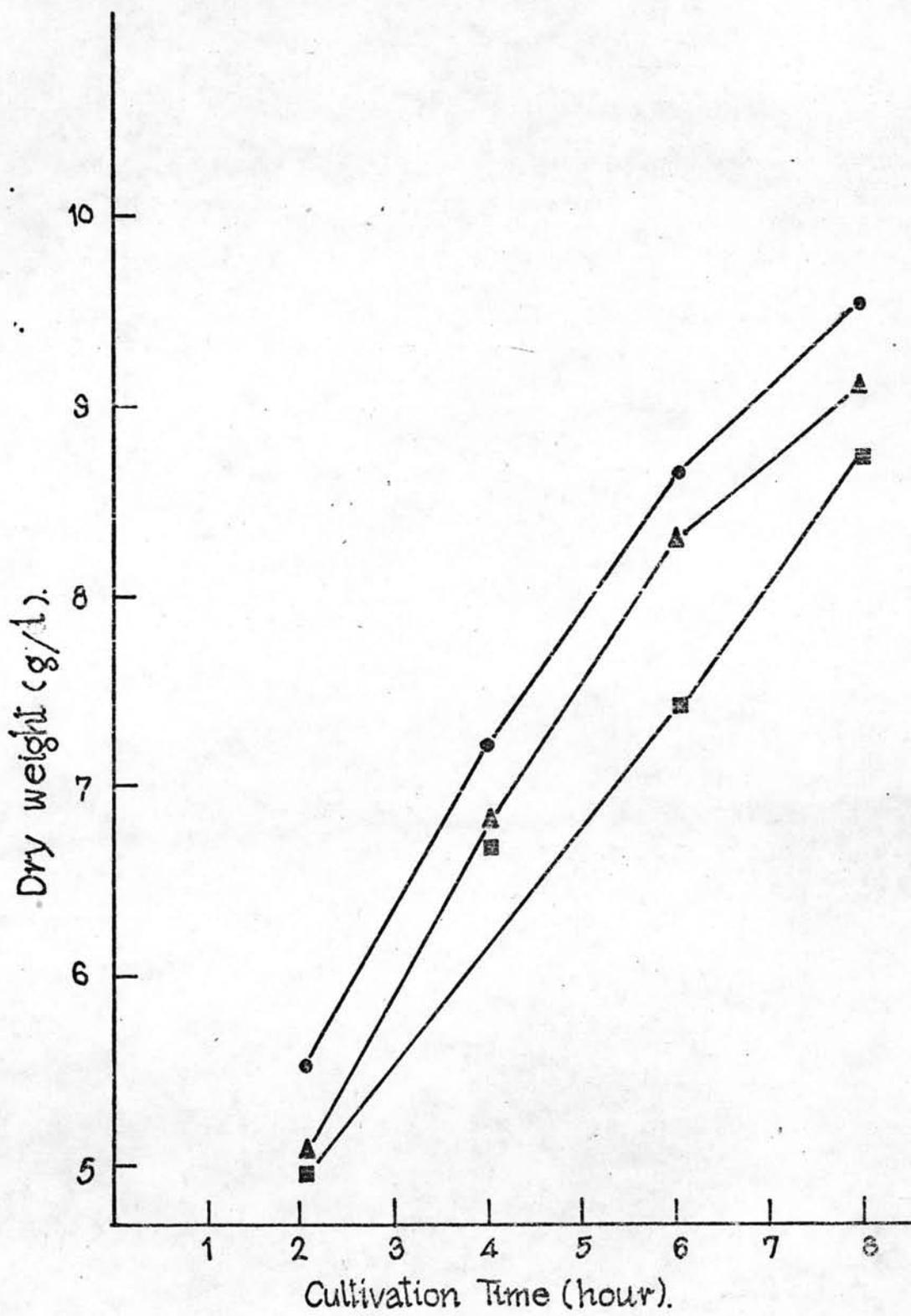


Figure 13

The relationship between the reduction of chemical oxygen demand (COD) and cultivation time during the growth of C. utilis in medium 1 at various aeration rates in stirred vessel fermenter.

Cultivation condition : see Figure 10

■ 0.5 VVM.

● 1 VVM.

▲ 1.5 VVM.

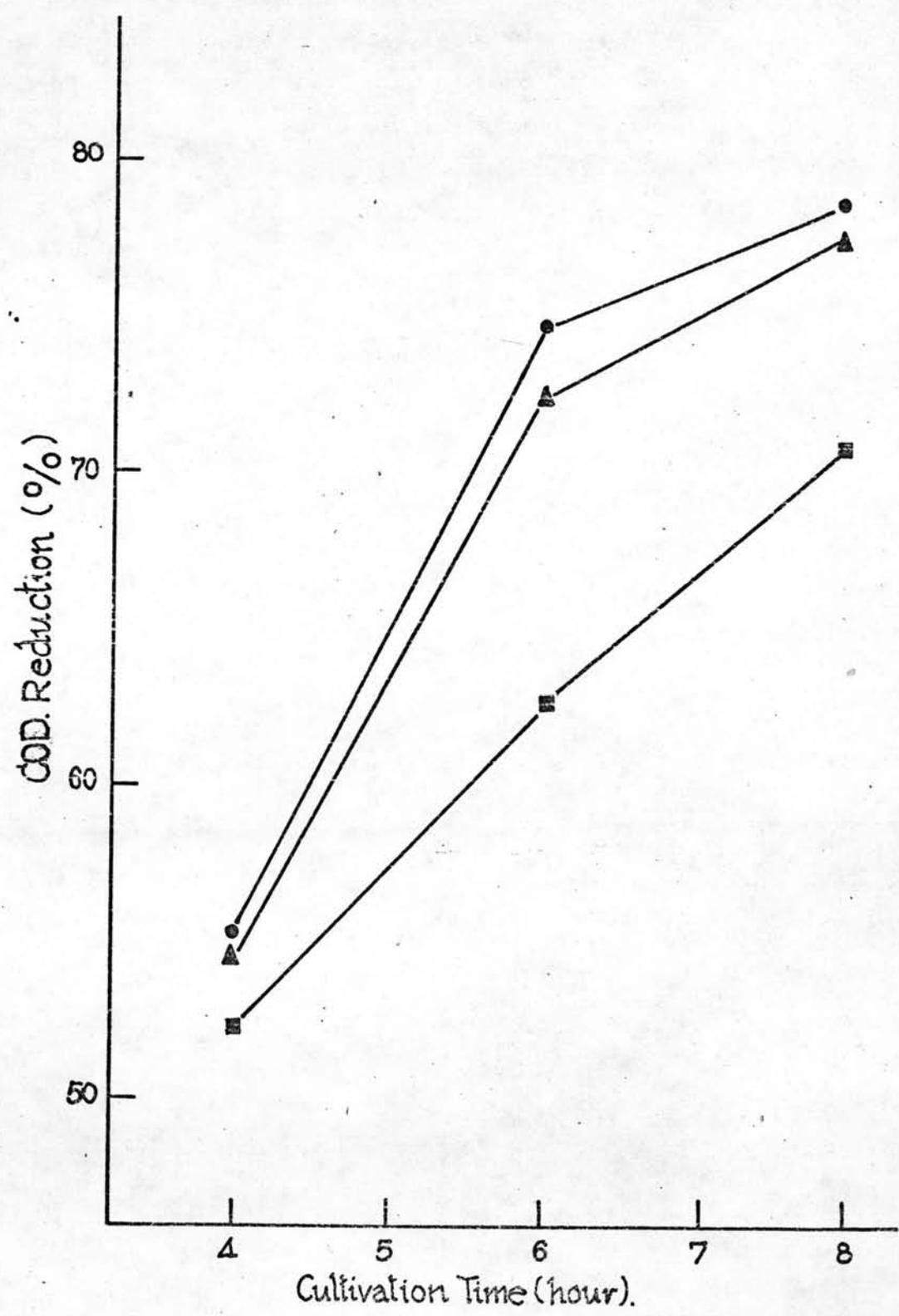


Figure 14

The relationship between the reduction of chemical oxygen demand (COD) and sugar consumption of C. utilis during the growth in medium 1 at various aeration rates in stirred vessel fermenter

Cultivation condition : see Figure 10

- 0.5 VVM.
- 1 VVM.
- ▲ 1.5 VVM.

