Chapter IV

RESULTS

4.1 Qualities of Pineapple Juice.

Some properties of fresh pineapple juice which related to the requirement of alcohol and acetic acid fermentation were analysed before the process was started.

The most prevalent of non-volatile organic acids occurring in pineapple juice is citric acid, it was determined by titration technique with NaOH. The average titratable acidity was 0.82 %. The average pH value of all samples varied from 3.8 to 4.0 with a little variability because pineapple juice is well buffered. The average degree Brix of pineapple juice was 16.2° and the average total invert sugars were 12.2 gm per 100 ml of pineapple juice.

The quality of pineapple juice varies gradually depending on many factors, for example: variety, nutrition, weather, season, and ripeness (41).

4.2 The Growth of S. ellipsoideus.

Indirect determination of the number of cells by the plate count technique and indirect determination of cell mass by using spectrophotometer were used to determine the growth of yeast. The results were shown in Figure 4 - 5 , it stated that after

the addition of inoculum to a new medium, there was gradually increase in the population of yeast cell during first five hours. Then the cells divided steadily from the fifth to tenth hour. Under this condition yeast cells were very active. After the tenth hour, the activities of yeast were decreased because of the exhaustion of some nutrients and the production of some toxic products such as malic, tartaric, and succinic acid during growth (12, 13). However, this did not represent the normal pattern of the growth but rather selected some portions of the normal growth curve, namely: the accelerated-growth phase, logarithmic phase, and nearly stationary phase.

4.3 Effect of Some Nutrients

Nutrients employed in this step were diammonium hydrogen phosphate and dipotassium hydrogen phosphate. The obtatined result was presented in Table 2 in term of average percent alcohol for each treatment after 24 hours. It could be explained that 1 % $(NH_4)_2HPO_4$, fortified into pineapple juice, gave no effect on the efficiency of alcoholic production. 1 % K_2HPO_4 gave higher yield of alcohol production than 1 % $(NH_4)_2HPO_4$ but lower than 0.5 % $(NH_4)_2HPO_4$ plus 0.5 % K_2HPO_4. However, in the further experiment only K_2HPO_4 was chosen as a nutrient for Saccharomyces ellipsoideus because $(NH_4)_2HPO_4$ gave a poor yield, a yellowish brown color, and ammonia odor to pineapple juice.

4.4 Effect of Nutrient Concentration.

Different concentrations of K_2HPO_4 varied from 0.25 to 2.5 % were fortified in pineapple juice. The obtained results were presented in Figure 6, it indicated that the low concentration of 0.25 % K_2HPO_4 was not enough for the utilization by the yeast during the fermentation and it gave a low yield of alcohol. The minimum concentration which gave maximum yield of alcohol was at the level of 0.5 %. So this suitable concentration was used in the further experiment. The results also shown that using excess or too high concentration (1.0 to 2.5 %) of nutrient would decrease the yield of alcohol production because excess K_2HPO_4 gave unsuitable condition, such as osmotic pressure and phosphorus concentration in the mash, for the growth and activity of the yeast.

4.5 Effect of Sugar Concentration.

Different concentrations of sucrose were used as substrate for yeast in alcoholic fermentation. The fermentation was carried out for 7 days and the results were collected in Figure 7. It indicated that percent alcohol produced were increased proportionately to the sugar level between 12.5° and 30.2° Brix. But the suitable level should be between 18° and 20° Brix because in this range, the efficiency of alcohol production was higher than the range between 24° and 27° Brix.

The results also showed that using too high sugar concentration, more than 30° Brix, it reacted adversely on the yeast or the alcohol produced inhibited the action of the yeast. The result of these effects was that the alcohol production was equal to or less than the samples which had lower sugar level.

4.6 Change of pH, °Brix, and Percent Alcohol

The constituents in pineapple juice were changed during the alcoholic fermentation. The results were presented in Figure 8-10, it indicated that pH changed from 4.5 to 3.9 within 40 hours of anaerobic fermentation, after that the pH value was nearly constant at the value of 3.9. This decrease in pH value was due to the production of some oranic acids such as malic and succinic acid by S. ellipsoideus (13). Meanwhile, degree Brix of the mash decreased as the rate of alcohol produced. The final degree Brix was 5.7° represented as soluble solid and small amount of remaining sugar in pineapple juice.

Rate of alcohol production was very rapid between the 20th and 50th hour. The fermentation was nearly complete after 75 hours of fermentation because of the lack of substrates and nutrients and production rate almost constant during this period.

4.7 The Growth of Acetobacter aceti.

Indirect determination of cell mass by using spectrophotometer was used through out this experiment. The number of cells

was indirectly determined by the plate count technique from the 4th to the 28th hour. At the end of this experiment the plate count technique was done again in order to study whether the medium was contaminated by others micro-organisms.

The experimental results were shown in Figure 11, it indicated that after the addition of inoculum to a new medium, there was gradually increase in the population of Acetobacter cells during first 20 hours. Then the cells divided steadily from the 20th to the 42th hour. Under this condition the culture was very active. After the 42th hour, the activities were decreased and nearly constant because of the lack of some nutrients and the production of toxic products. At the end of this step, the results by pour plate technique indicated that there was no any contamination during the experiment.

4.8 Effect of Recycling on Acetic Acid Fermentation.

The fermented pineapple liquor was allowed to recirculated by trickling through the column several times. The acidity and the flow rate were checked during each cycle. Figure 12 showed the obtained results. Total acidity increased proportionately to the number of recycle until the fifth cycle or 60 hr. The fermentation reached the maximum level at this cycle. Then percentage of acid diminished gradually during the sixth cycle due to the volatilization and the oxidation of acetic acid to carbon dioxide and water by the species of A. aceti (3).

4.9 Effect of Phosphate on Acetic Acid Fermentation.

Since the level of phosphates in the pineapple juices was found to be quite low (41), it was decided to examine the effect of supplementing the fermented liquor with phosphate. Only 0.5 % of K_2HPO_4 was supplemented in the fermented liquor. The experiment followed the above procedure and the results were collected in Figure 13. The activity of the Acetobacter culture and the amount of acetic acid were enhanced remarkably. The maximum yield was 5.84 % of acetic acid after only 48 hours of fermentation or after the fourth cycle. Comparing to the normal fermentation, the phosphate supplemented one gave higher efficiency and percentage of acetic acid.

4.10 Effect of Acidification.

Cruess (11) commented that S. ellipsoideus ceased to grow when the concentration of acetic acid exceeded 0.5 % in the fermented liquor. Therefore, the vinegar industry followed the Cruess's suggestion by decreasing the pH of the juice with vinegar prior to the fermentation. In this experiment, acetic acid was added to 1000 ml fermented pineapple juice to give initial acidity 1.33 % and also supplemented with 0.5 % phosphate. The fermented juice was then allowed to trickle through the generator column charged with the Acetobacter cultures as outlined previously. The results were represented in Figure 14, it indicated that the acidification of fermented liquor with acetic acid

further improved the activity of the Acetobacter culture and increased both the yield of acetic acid (7.48 %) and the efficiency of the fermentation. Again the maximum acid production was achieved after the fourth cycle (48 hr).

4.11 Effect of Air Flow Rates.

Three different air flow rates of 0.035, 0.053, and 0.106 vvm. were introduced into the generator column. The interesting results were obtained and collected in Figure 15. It indicated that the yields of acetic acid decreased from 5.56 % to 4.82 % and to 3.58 % when the flow rate of air varied from 0.035 to 0.053 and to 0.106 vvm. respectively.

4.12 Effect of Fermented Juice Flow Rates.

Two flow rates of fermented liquor were done at 1000 cm^3/12 hr and 1500 cm^3/12 hr. The results, showed in Figure 16, indicated that the first flow rate gave the maximum yield at the fifth cycle or 60 hours and the latter took longer time and the maximum yield would receive after the seventh cycle or 84 hours because its resident time was shorter. The result also showed that the yield of acid, using higher flow rate, was slightly increased.

Table 2 Effect of nutrients $(NH_4)_2HPO_4$ and K_2HPO_4 on the utilization of pineapple juice for alcoholic fermentation by S. ellipsoideus.

No.	Nutrients	Refractive-Index[1] of Distillate	% Alcohol
1	Non	1.3350	3.18
2	$(NH_4)_2HPO_4$ 1 %	1.3350	3.18
3	K_2HPO_4 1 %	1.3351	3.40
4	$(NH_4)_2HPO_4$ 0.5 % + K_2HPO_4 0.5 %	1.3355	4.15

[1]Average refractive - index of 2 samples at 25°C and fermenting time 24 hours.

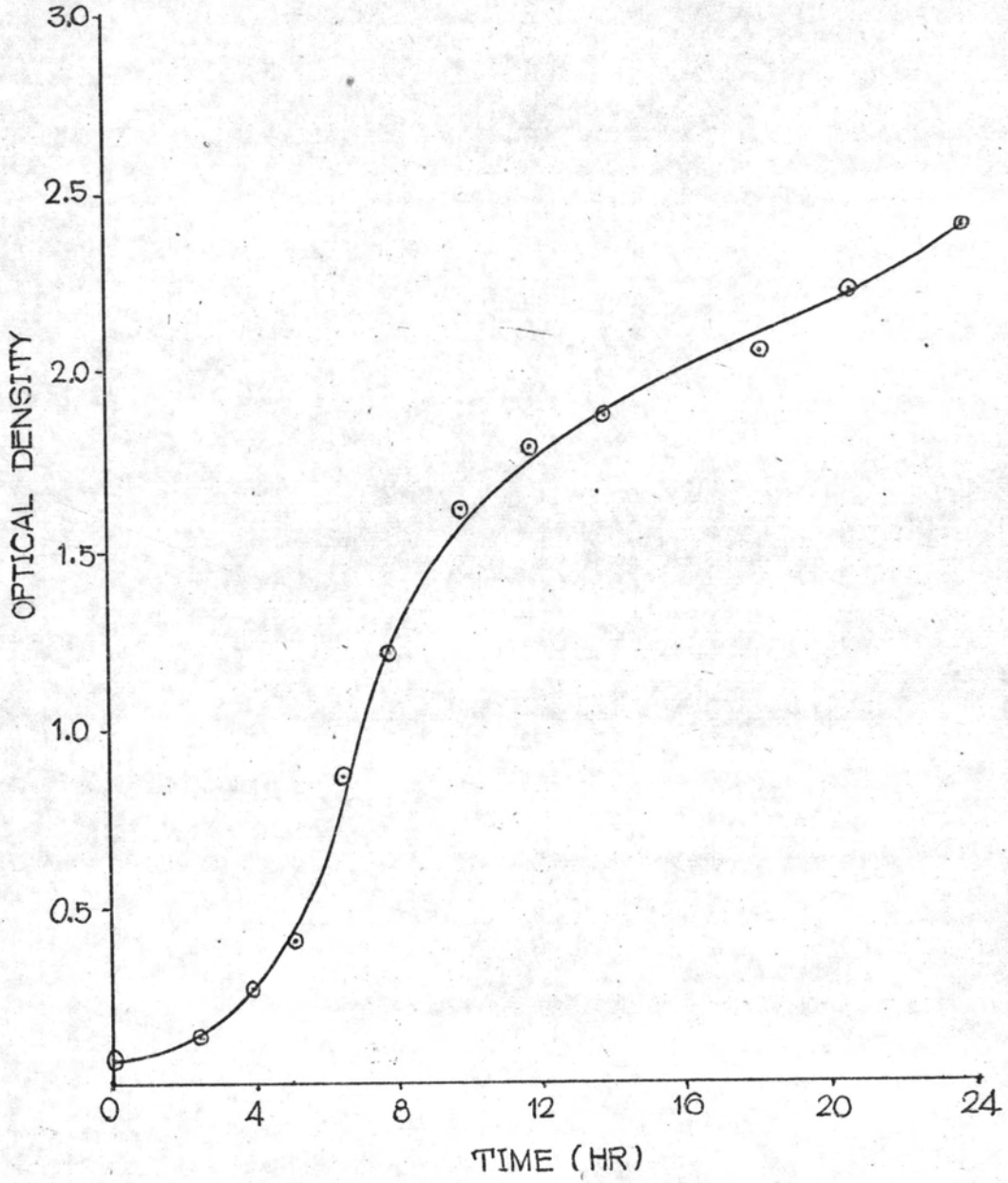

Fig.4 The growth of Saccharomyces ellipsoideus within 24 hours in pineapple juice medium. Results are represented in the term of average optical density of two samples in 500 ml shake flasks. The optical density was measured at 500 nm. and the speed of skaking machine was 240 rpm.

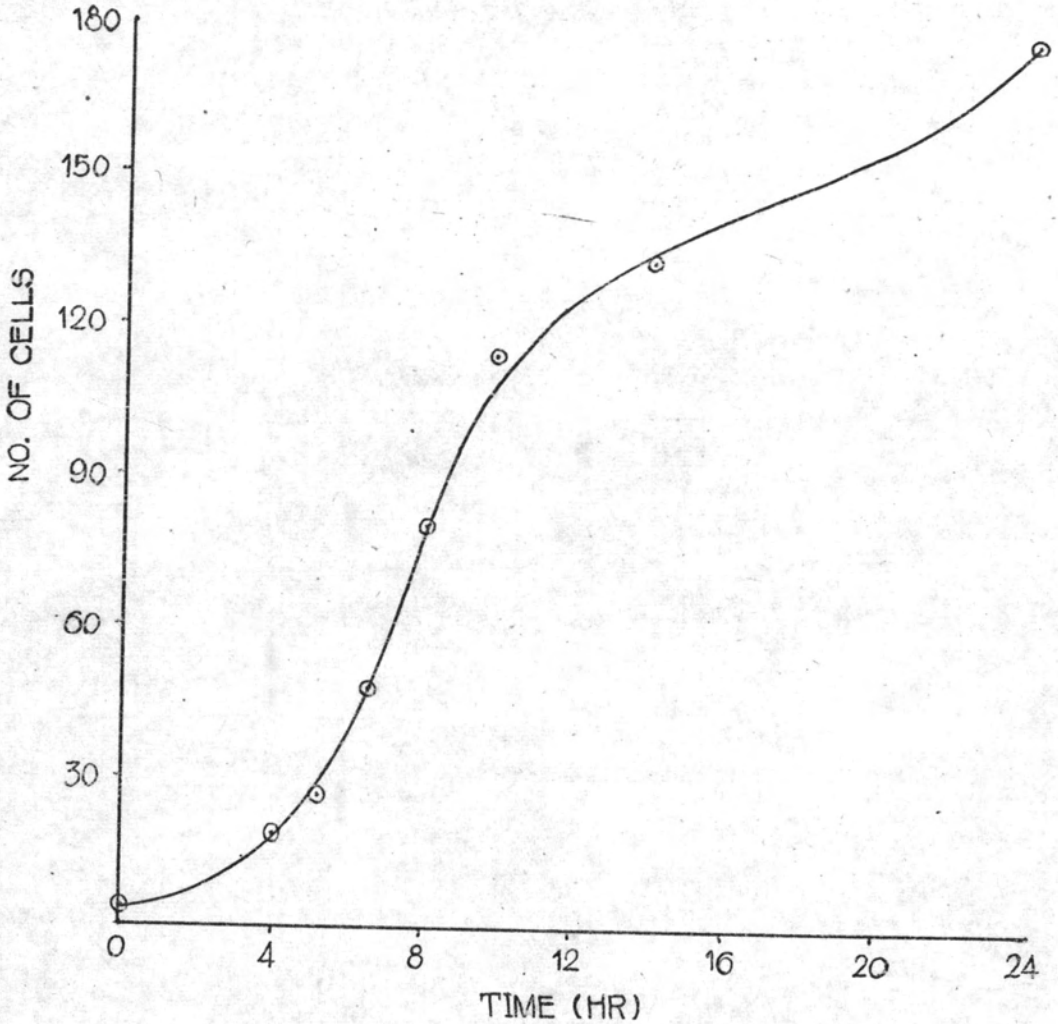

Fig.5 The growth of Saccharomyces ellipsoideus within 24 hours in pineapple juice medium. Results are represented in term of average number of cells of two samples in 500 ml shake flasks at 240 rpm.

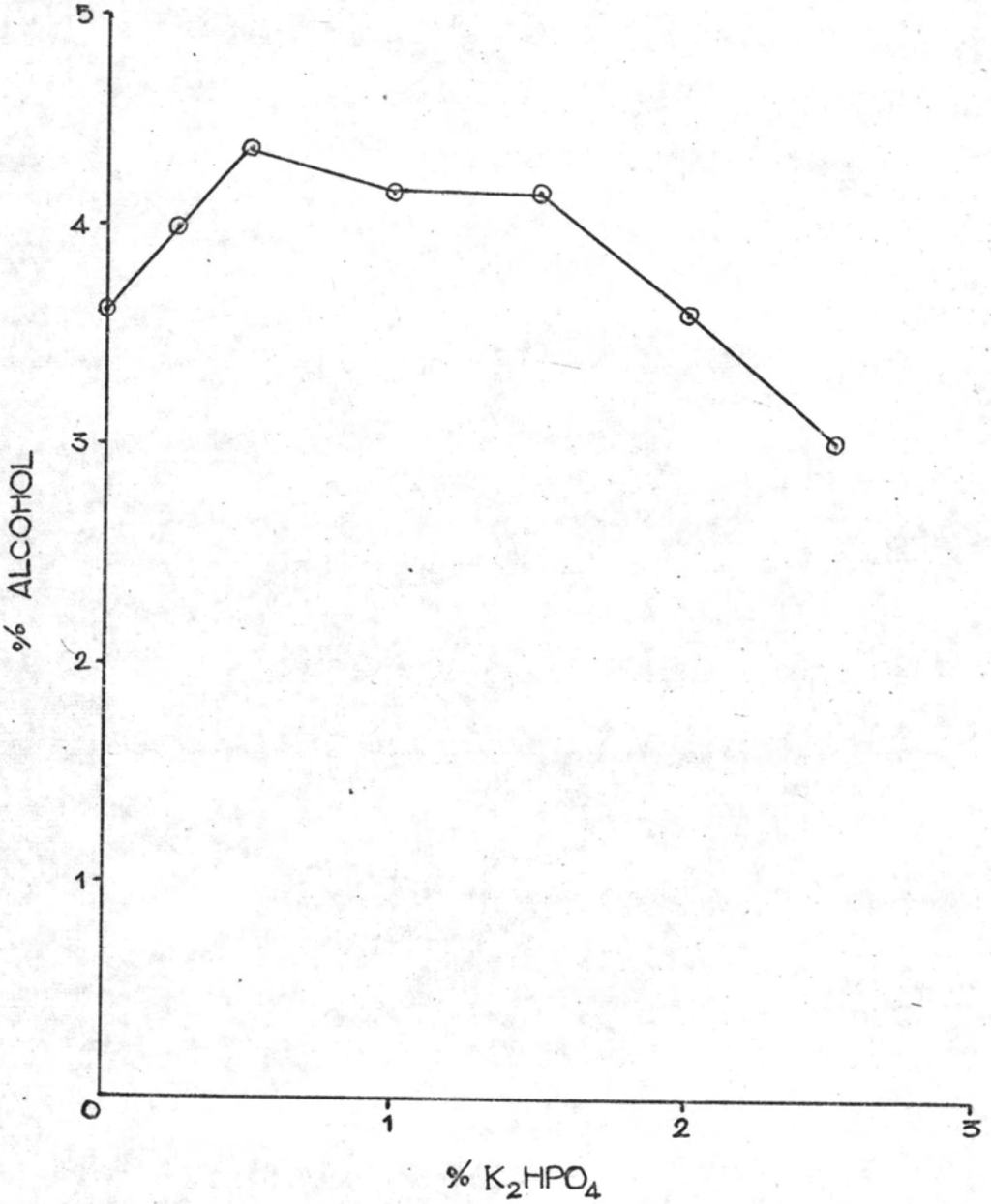

Fig.6 Effect of concentrations of nutrient, K_2HPO_4, on the utilization of pineapple juice for alcoholic fermentation by Saccharomyces ellipsoideus. Resultsare represented in term of average percent alcohol within 24 hours of fermentation. Initial total soluble solid of pineapple juice was 22° Brix and pH 4.5 with 5 percent inoculum.

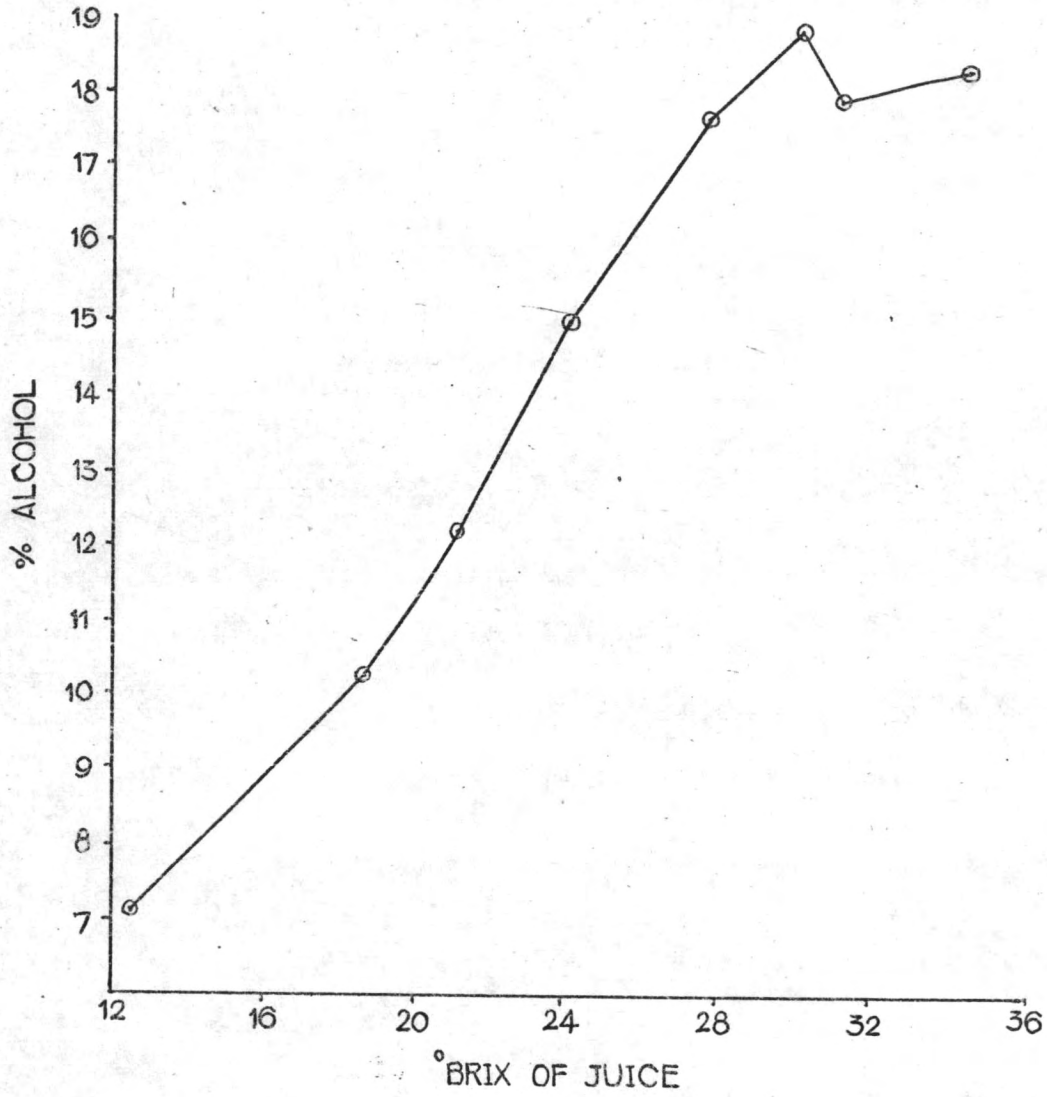

Fig.7 Effect of sugar levels on the utilization of pineapple juice for alcoholic fermentation by Saccharomyces ellipsoideus. Results are represented in term of average percent alcohol within 7 days of fermentation. Initial pH value of pineapple juice was 4.5 with 5 % inoculum and 0.5 % K_2HPO_4.

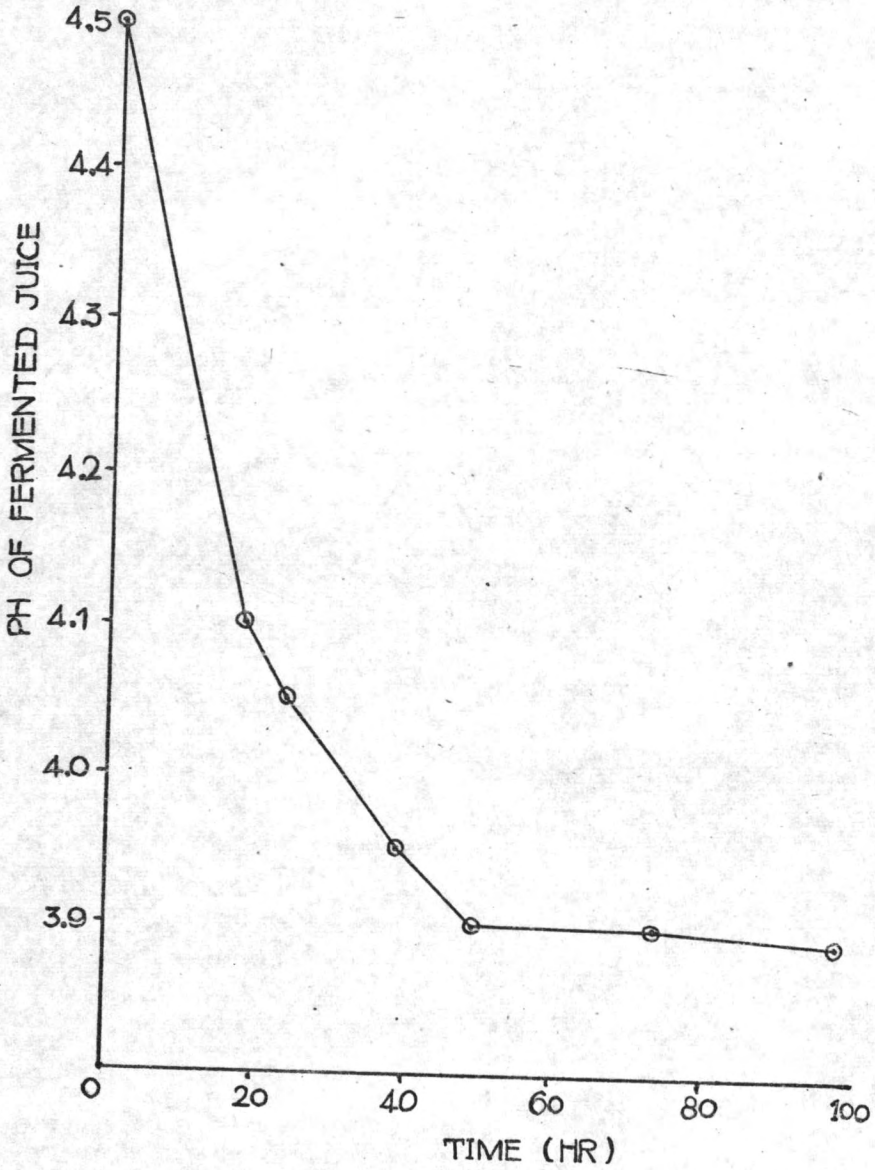

Fig.8 Change of pH values during the alcoholic fermentation of pineapple juice by Saccharomyces ellipsoideus. The pH values were measured at various time intervals within 4 days. Initial pH values was 4.5 and total soluble solid of I9.6 degree Brix.

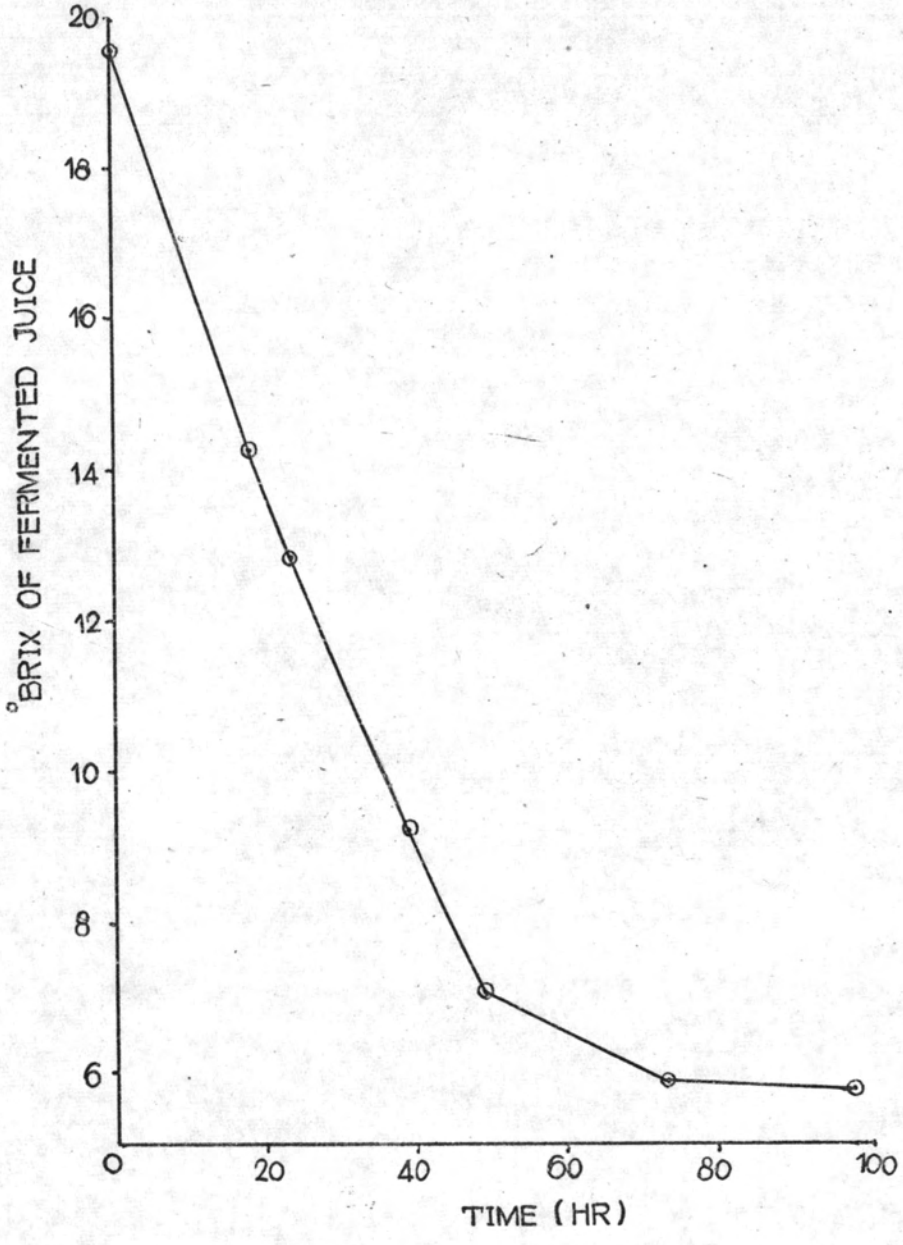

Fig.9 Change of sugar levels during the alcoholic fermentation of pineapple juice by Saccharomyces ellipsoideus. Results are represented in term of degree Brix by using Abbé Refractometer. Initial degree Brix was 19.6° and pH at 4.5.

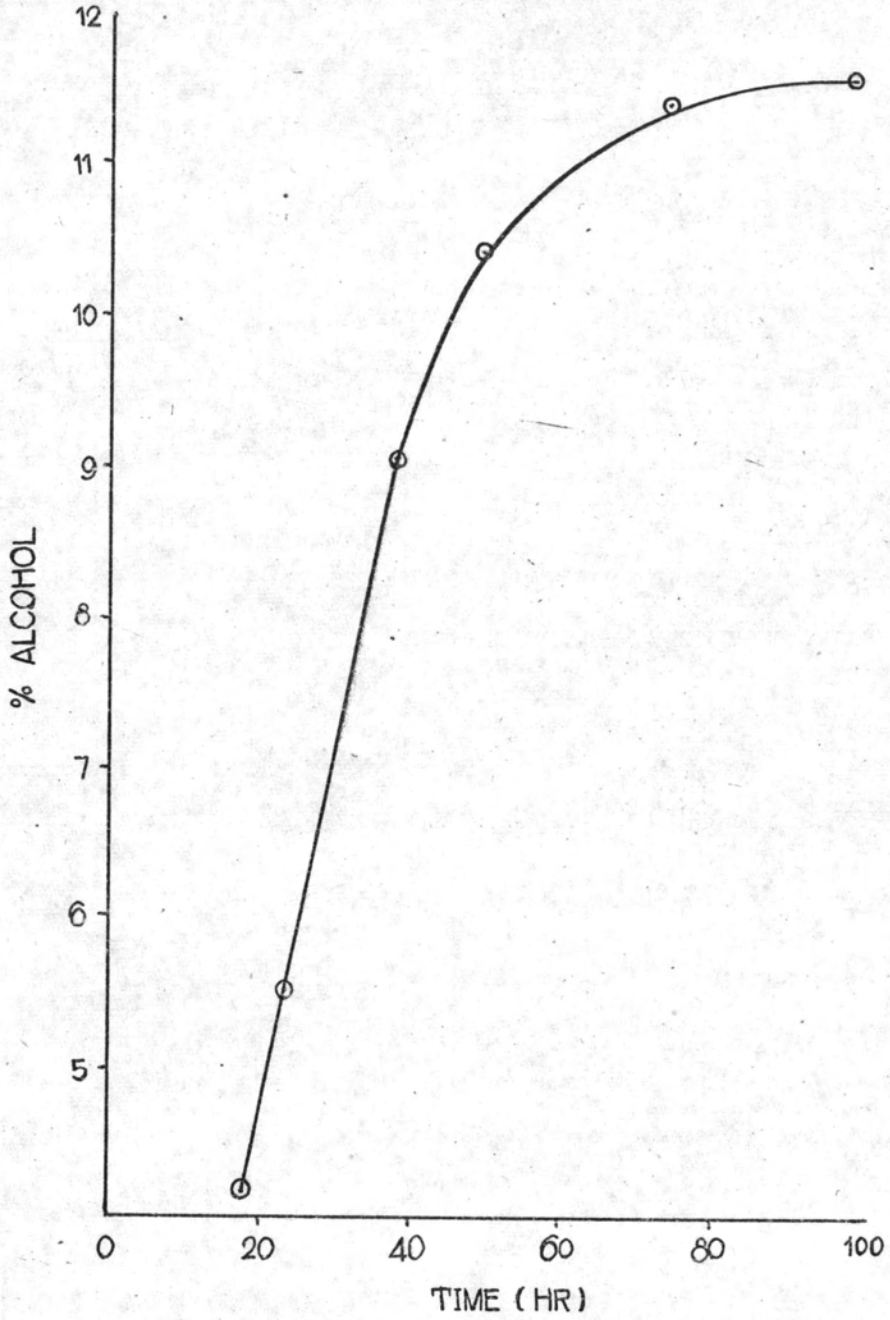

Fig.10 Change of percent alcohol during alcoholic fermentation of pineapple juice by Saccharomyces ellipsoideus. Initial degree Brix of pineapple juice was 19.6° and pH at 4.5.

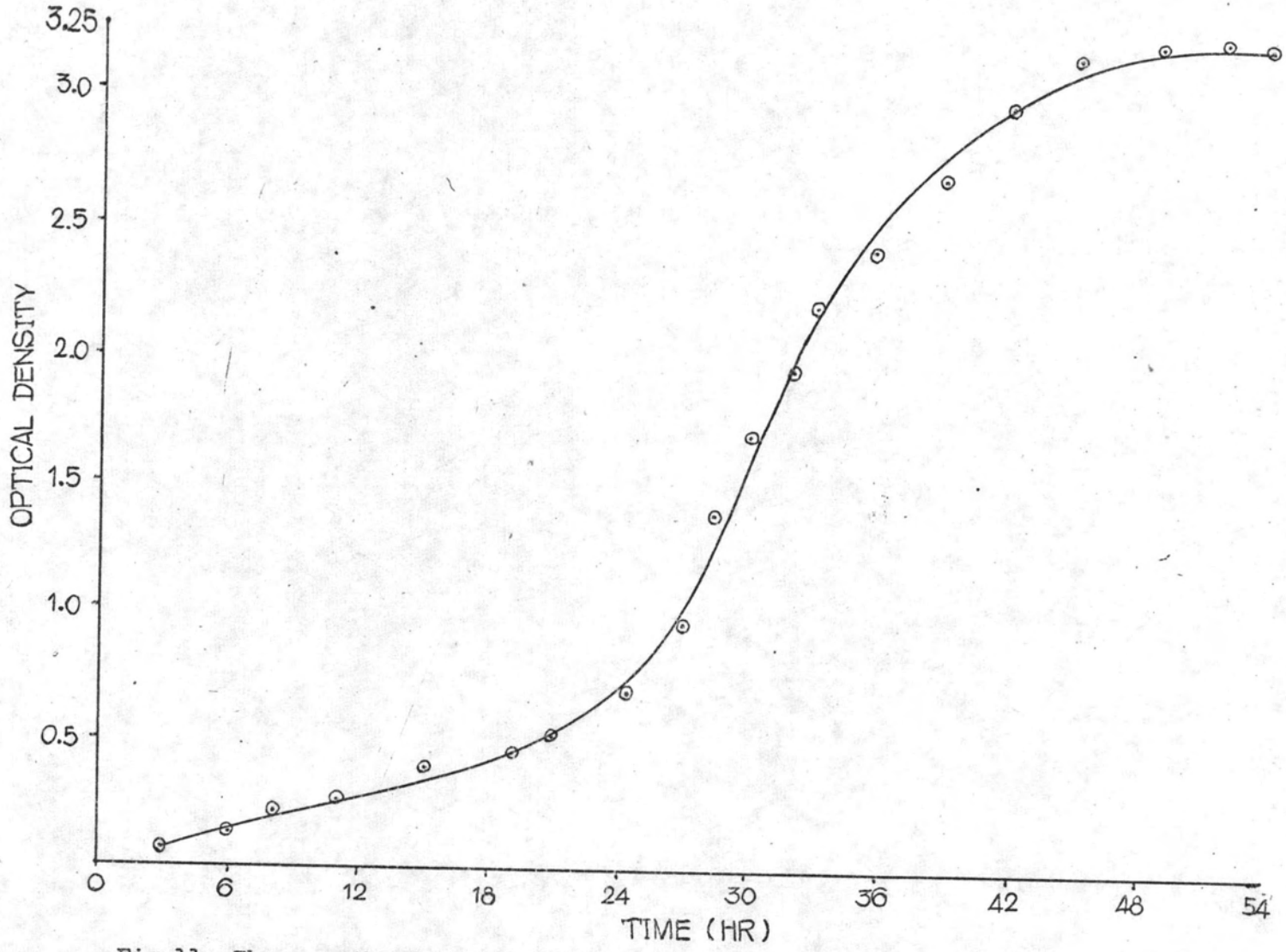

Fig.11 The growth of Acetobacter aceti within 48 hours in Acetobacter broth. Results are represented in the term of average optical density of two samples at 500 nm.

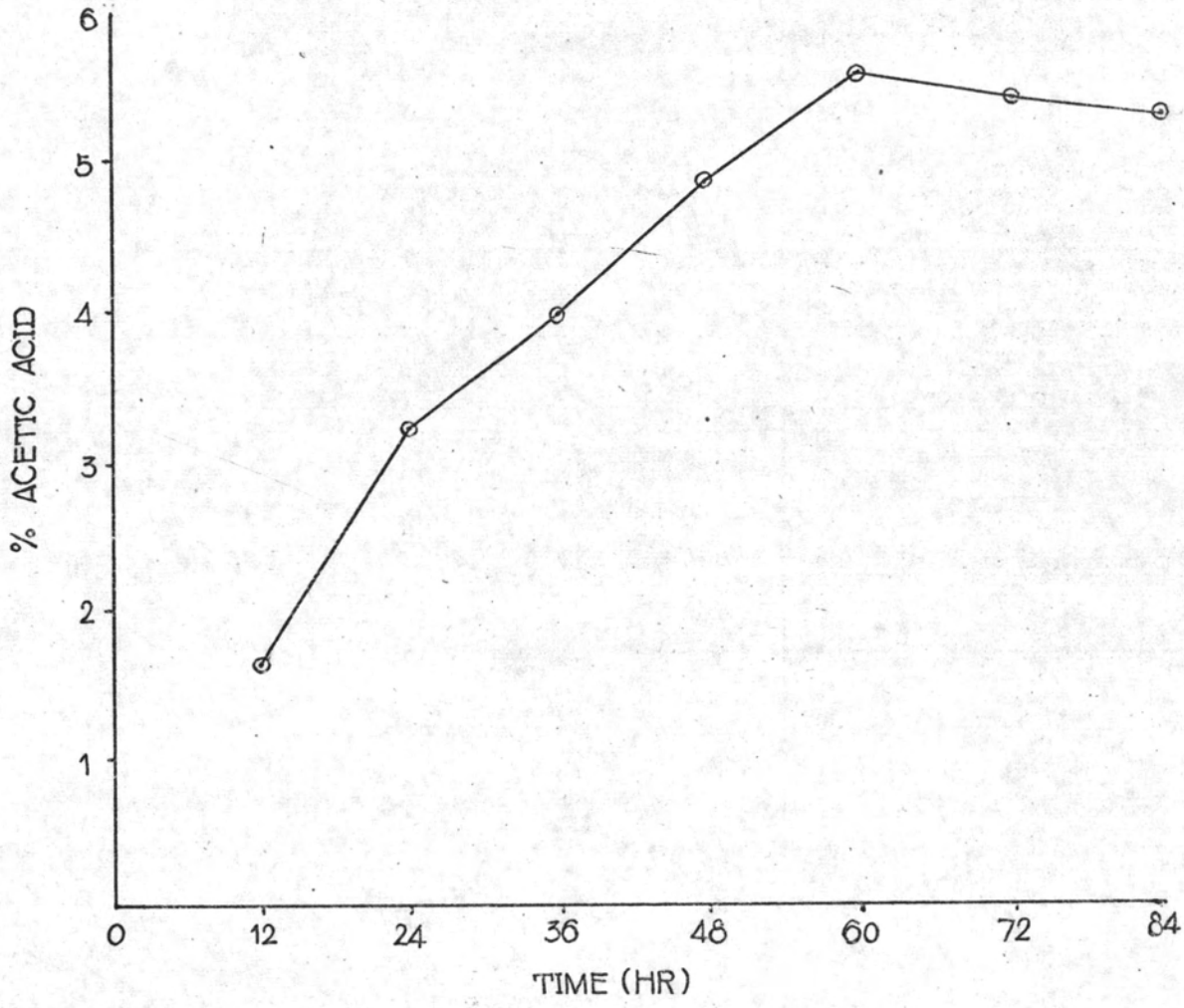

Fig.12 Effect of recycling on the rate and efficiency of acetic acid fermentation in generator column using Acetobacter aceti and fermented pineapple juice. Results are reported as percent acetic acid formed.

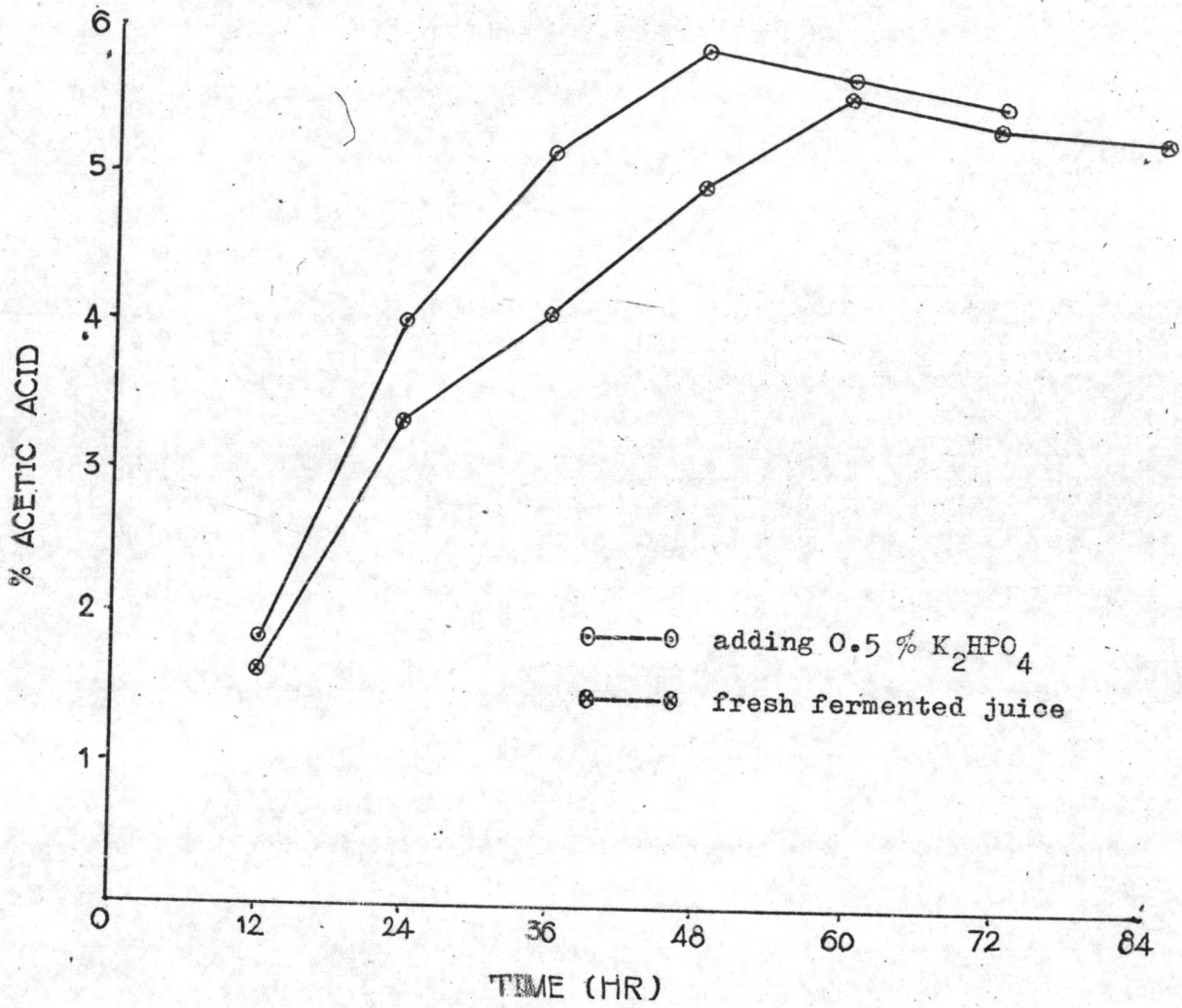

Fig.13 Effect of adding 0.5 % K_2HPO_4 to the fermented pineapple juice on the rate and efficiency of acetic acid fermentation in generator column by Acetobacter aceti. Results are reported as percent acetic acid formed.

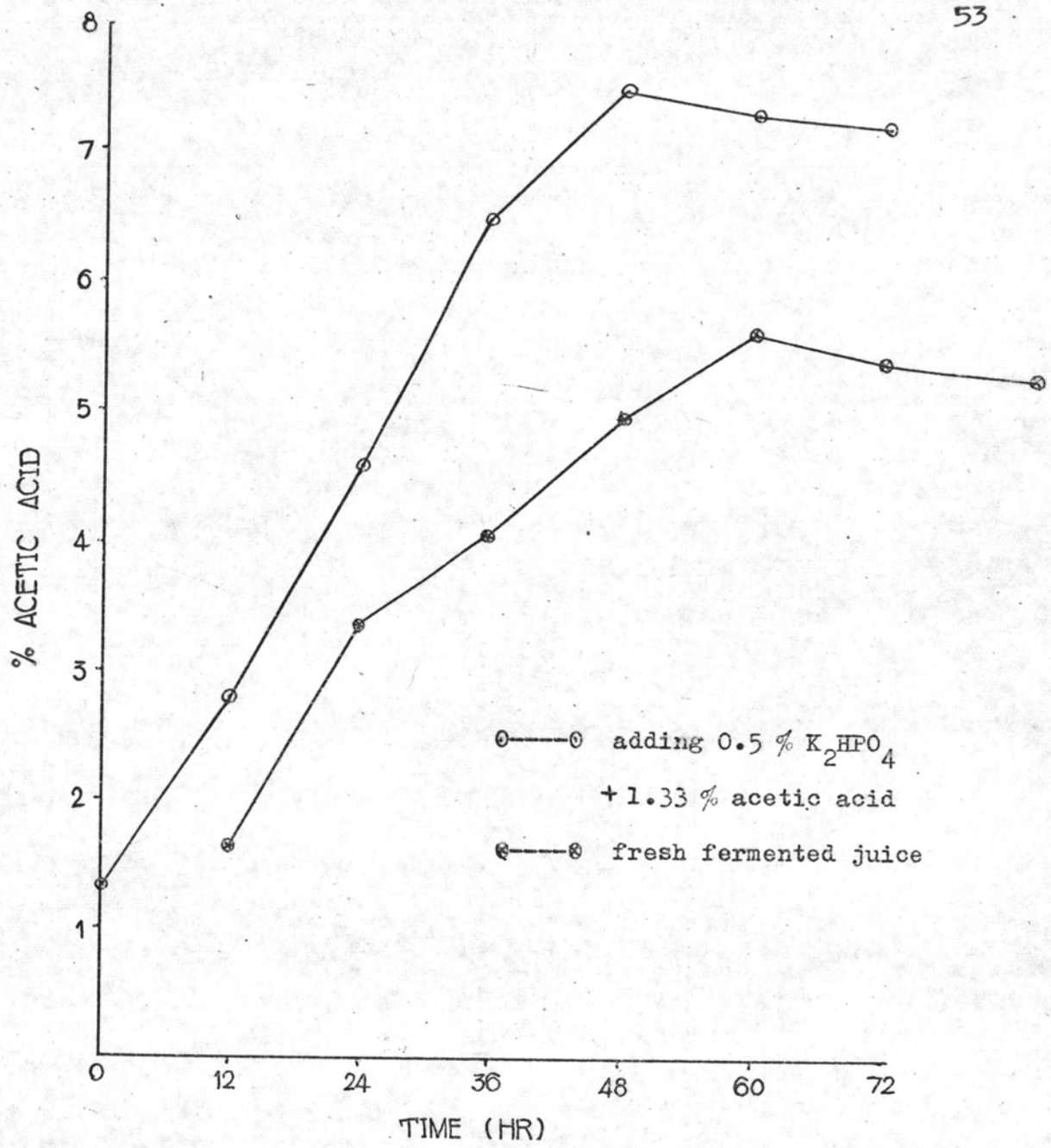

Fig.14 Effect of adding 1.33 % acetic acid and 0.5 % K_2HPO_4 to the fermented pineapple juice on the rate and efficiency of acetic acid fermentation in generator column by Acetobacter aceti. Results are reported as percent acetic acid formed.

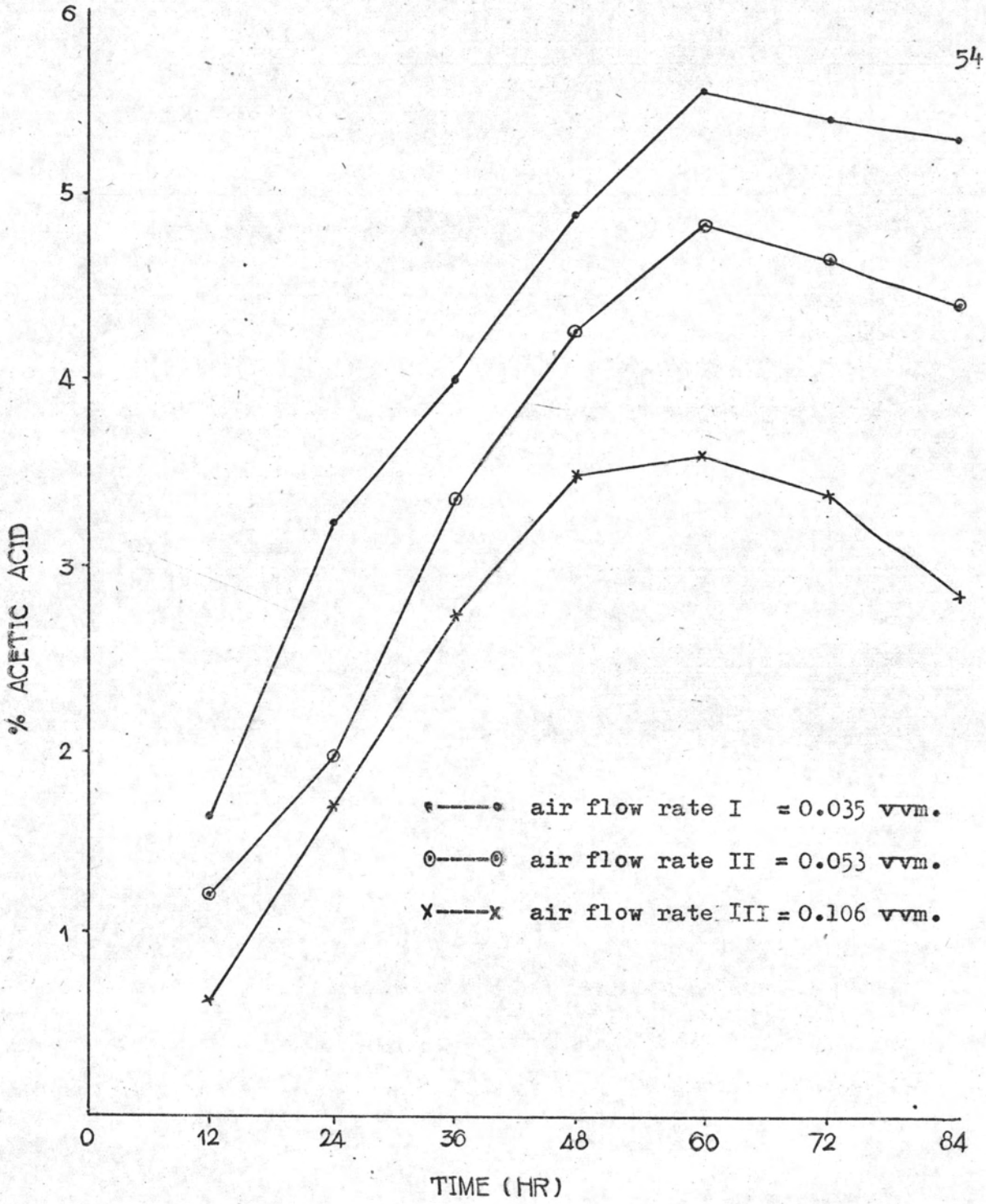

Fig.15 Effect of air flow rates on the rate and efficiency of acetic acid fermentation in generator column using Acetobacter aceti and fermented pineapple juice. Results are reported as percent acetic acid formed.

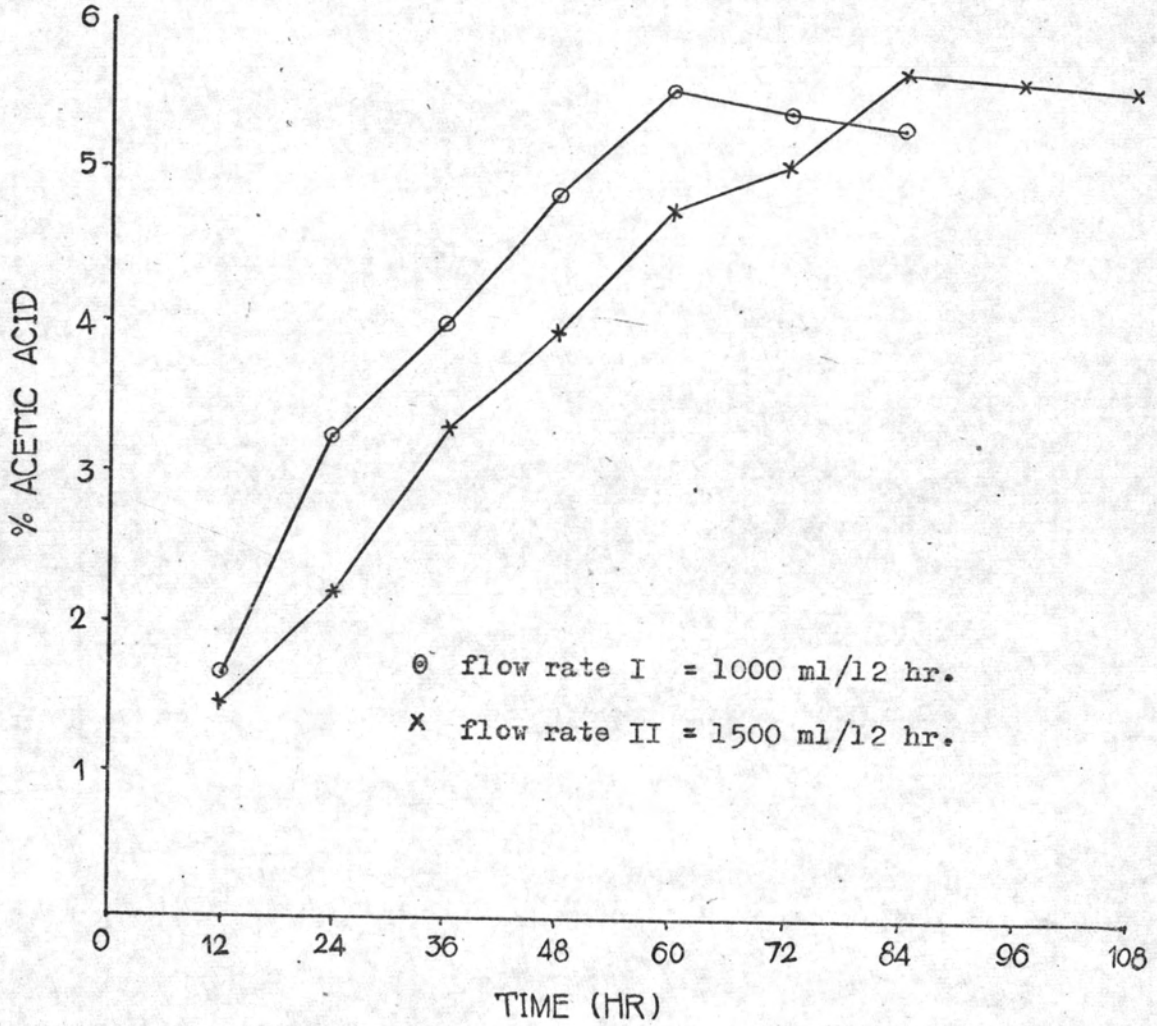

Fig.16 Effect of fermented juice flow rates on the rate and efficiency of acetic acid fermentation in generator column using Acetobacter aceti and fermented pineapple juice. Results are reported as percent acetic acid formed.