LVIII. THE INFLUENCE OF THE FATTY ACIDS AND THEIR SALTS ON ALCOHOLIC FERMENTATION BY LIVING YEAST.

PART I. ACETIC AND FORMIC ACIDS AND THEIR SODIUM, POTASSIUM AND AMMONIUM SALTS.

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In order to ascertain what effect a given substance has on the fermentation of sugar by living yeast, it is necessary in the first place to decide on an appropriate standard method of determining the normal rate of fermentation. When a sugar solution is treated with yeast the liquid gradually increases in acidity and a variable rate is observed. The use of a buffer is therefore essential and the question then arises how variation in the concentration and composition of the buffer affects the rate of fermentation. The following experiments were instituted in order to obtain some satisfactory basis for the comparison of the action of various salts, etc., which have from time to time been stated to have an accelerating or depressing effect on the rate of fermentation by living yeast.

The relation between rate of fermentation and p_H value has been a matter of research for many years. Many authors have already discussed the question whether the rate of fermentation reveals any remarkable difference between the various acids employed to regulate the p_H value of the medium. Hägglund [1914] tested Bial's conclusions [1902] as to various acids, and pointed out the relation between the influence of an acid upon the rate of fermentation and the degree of dissociation of the acid. On the other hand, Euler and Heintze [1919] observed that weak acids such as oxalic, chromic and acetic acids have a marked specific poisoning effect on yeast above a certain concentration.

Recently Hägglund and Augustson [1925, 1, 2] have worked with buffer solutions composed of acetic acid-acetate, lactic acid-lactate and phosphoric acid-phosphate, and observed that the optimum p_H value depends upon the variety of sugar, period of observation and kind of acid. They also noted that yeast is very sensitive to free acetic acid.

In the present experiments the effects of the salts of acetic and formic acids on the rate of fermentation by living yeast have been studied with the special object of ascertaining the influence of concentration of hydrogen ions, and the nature and concentration of the free acids and their salts.

Methods.

The apparatus described by Harden, Thompson and Young [1910] was used. 20 cc. of 10 % glucose solution and 20 cc. of the various buffer solutions were placed in a 50 cc. flask, saturated with CO_2, connected with the mercury burette, and incubated in a water-bath at 25°. 10 cc. of the solution was pipetted out, and its p_H value was colorimetrically observed by the capillator method. 2 g. of pressed top yeast were added to the remaining 30 cc. of solution and the amount of evolved CO_2 was observed at intervals of 5 minutes for nearly 1 hour. The liquid was then filtered, the filtrate saturated with CO_2, incubated, and the p_H value again determined by the above method. For the rate of fermentation, the average number of cc. of CO_2 evolved in 5 minutes was chosen. In order to allow for variation in the fermenting power of different samples of yeast, a fermentation was always carried out in presence of a 0·2 *M* acetate solution at p_H 4·7, which was made by mixing two volumes of 0·2 *M* sodium acetate and one volume of 0·2 *M* acetic acid. The standard rate of fermentation under these circumstances was taken as 2·4 cc. per 5 minutes. When the observed rate differed from this, the ratio of the two numbers was found and used as a factor to correct all the observations of the series to the standard.

In order to test the validity of this method a series of experiments was made in which a number of different samples of yeast, or the same sample after being kept for various periods, were allowed to ferment the same series of solutions.

Exp. 1. Four solutions were used, made by mixing 0·2 *M* potassium acetate and 0·2 *M* acetic acid, and compared with the standard made up as described above with two parts of 0·2 *M* sodium acetate and one part of 0·2 *M* acetic acid. The concentration in every case is therefore 0·2 *M* and the solutions were as follows:

No. of solution	Ratio of acetate/acetic acid	Mean rate of fermentation cc. per 5′	Same yeast after keeping		
			For 24 hours at 0°	For 2 days at 0°	For 3 days at 0°
1 K acetate	2 : 5	0·02	0·17	0·2	0·12
2 „	2 : 1	2·6	2·9	2·6	2·65
3 „	5 : 1	6·1	6·25	6·5	6·4
4 „	1 : 0	8·24	8·25	7·6	7·8
5 Na acetate	2 : 1	1·76	1·87	1·86	1·78

The p_H values did not alter during the fermentation.

Exp. 2. In this case a different sample of yeast was used with the same set of solutions.

	Number of solution				
	1	2	3	4	5
I. Fresh yeast	0·05	1·75	4·6	6·0	1·1
II. After keeping for 1 day	0·1	1·97	4·5	6·2	1·4
III. „ „ „ 2 days	0·14	1·8	4·0	5·7	1·3
IV. „ „ „ 3 „	0·16	1·6	3·9	5·5	1·2

Taking the values for solution No. 5 as standard and making the rate in each case equal to 1 we get the following relative values by dividing the value for each solution by that of the standard (No. 5) in each series.

	Exp. 1. Number of solutions					*Exp.* 2. Number of solutions				
Series	1	2	3	4	5	1	2	3	4	5
I	0·01	1·48	3·47	4·68	1	0·04	1·56	4·04	5·33	1
II	0·09	1·55	3·34	4·41	1	0·07	1·42	3·27	4·44	1
III	0·11	1·40	3·36	4·09	1	0·11	1·36	4·05	4·27	1
IV	0·07	1·49	3·52	4·38	1	0·13	1·33	3·19	4·49	1
Average	0·07	1·48	3·42	4·39	1	0·09	1·42	3·64	4·63	1

It will be seen that the values in the various series and the average values for the two different samples of yeast agree very well, although the actual rates observed with the second sample of yeast were considerably lower than with the first.

Concentration of free acid.

The concentration of free acid in the medium is equal to that added + the negligibly small amount formed by hydrolysis of the salt + the amount of acid produced from the salt in the process of saturation with CO_2. In order to ascertain the last amount, the following experiments were carried out.

To 30 cc. of mixtures having the same composition as the solutions used in fermentation, 10 cc. of HCl of suitable strength was added after saturation with CO_2, and the evolution of CO_2 was measured in the same apparatus as was used for the fermentations. The results have not been corrected for any possible error due to the altered solubility of CO_2 in the solutions after mixing, but this is not likely to be appreciable. From the amount of CO_2 thus obtained the total quantity of free acid can be easily calculated by using the equation:

$$CH_3COONa + H_2CO_3 = CH_3COOH + NaHCO_3.$$

A single example is given below of the case of sodium acetate to which no free acetic acid has been added. The results obtained are incorporated in Table I.

Concentration of Na acetate (*M*)	0·5	0·2	0·1	0·05	0·025
Concentration of HCl (*M*) added	2·0	0·8	0·4	0·2	0·1
cc. of CO_2 evolved (N.T.P.)	12·19	5·56	4·39	2·6	1·79
Concentration of free acetic acid (*M*)	0·018	0·008	0·006	0·004	0·003

Results.

Table I gives the results of the experiments.

Discussion.

The curves (Fig. 1) when the rate of fermentation is plotted against the p_H are very similar for equal concentrations of the buffer solutions composed of acetic acid and Na acetate, K acetate and NH_4 acetate respectively. With these acetate-acetic acid buffer solutions, the rate of fermentation at constant

Table I.

A. *Sodium acetate and acetic acid.*

Ratio (Salt/Acid)	Concentration of total acetate (*M*)	Concentration of $C_2H_4O_2$ (*M*)	Corrected rate of fermentation cc. per 5′	*p*H Beginning	*p*H End
Exp. 1.					
2 : 5	0·5	0·357	0·04	4·1	4·1
2 : 1	0·5	0·167	0·7	4·7	4·7
5 : 1	0·5	0·085	2·5	5·1	5·1
1 : 0	0·5	0·018	5·7	6·2	6·2
2 : 1 (standard)	0·2	—	2·4	4·7	4·7
Exp. 2.					
2 : 5	0·2	0·143	0·2	4·0	4·0
2 : 1	0·2	0·067	2·4	4·7	4·7
5 : 1	0·2	0·034	7·3	5·1	5·1
1 : 0	0·2	0·008	9·1	6·0	5·9
Exp. 3.					
2 : 5	0·1	0·071	1·4	4·0	4·0
2 : 1	0·1	0·033	6·5	4·7	4·7
5 : 1	0·1	0·018	8·8	5·0	5·0
1 : 0	0·1	0·006	10·8	5·7	5·4
Exp. 4.					
2 : 5	0·05	0·036	4·8	4·0	4·0
2 : 1	0·05	0·017	8·1	4·7	4·6
5 : 1	0·05	0·009	10·5	5·0	4·8
1 : 0	0·05	0·004	11·6	5·4	5·0
Exp. 5.					
2 : 5	0·025	0·018	7·0	4·0	3·8
2 : 1	0·025	0·008	9·3	4·6	4·3
5 : 1	0·025	0·005	10·1	4·9	4·5
1 : 0	0·025	0·003	11·4	5·4	4·7

B. *Potassium acetate and acetic acid.*

Ratio (Salt/Acid)	Concentration of total acetate (*M*)	Concentration of $C_2H_4O_2$ (*M*)	Corrected rate of fermentation cc. per 5′	*p*H Beginning	*p*H End
Exp. 6.					
2 : 5	0·5	0·357	0·01	4·2	4·2
2 : 1	0·5	0·167	0·9	5·0	5·0
5 : 1	0·5	0·085	3·1	5·3	5·3
1 : 0	0·5	0·025	6·0	6·2	6·2
Exp. 7.					
2 : 5	0·2	0·143	0·3	4·2	4·2
2 : 1	0·2	0·067	2·3	4·9	4·9
5 : 1	0·2	0·035	5·0	5·3	5·3
1 : 0	0·2	0·013	8·6	6·1	5·8
Exp. 8.					
2 : 5	0·1	0·071	2·3	4·1	4·1
2 : 1	0·1	0·033	7·1	4·8	4·8
5 : 1	0·1	0·018	9·0	5·2	5·2
1 : 0	0·1	0·007	10·2	5·8	5·6
Exp. 9.					
2 : 5	0·05	0·036	5·7	4·1	4·0
2 : 1	0·05	0·017	8·4	4·8	4·6
5 : 1	0·05	0·009	9·8	5·2	4·9
1 : 0	0·05	0·005	10·4	5·6	5·2
Exp. 10.					
2 : 5	0·025	0·018	7·5	4·1	3·7
2 : 1	0·025	0·008	9·6	4·7	4·5
5 : 1	0·025	0·005	10·1	5·0	4·7
1 : 0	0·025	0·002	10·1	5·4	4·9

C. *Ammonium acetate and acetic acid.*

Exp. 11.					
2 : 5	0·5	0·357	0·02	4·0	4·0
2 : 1	0·5	0·167	0·2	4·7	4·7
5 : 1	0·5	0·084	1·1	4·9	4·9
1 : 0	0·5	0·013	6·9	6·2	6·1
Exp. 12.					
2 : 5	0·2	0·143	0·2	4·0	4·0
2 : 1	0·2	0·067	1·6	4·6	4·6
5 : 1	0·2	0·033	3·6	4·9	4·9
1 : 0	0·2	0·007	10·0	5·9	5·7
Exp. 13.					
2 : 5	0·1	0·071	2·0	3·9	3·9
2 : 1	0·1	0·033	6·2	4·6	4·6
5 : 1	0·1	0·017	9·7	5·0	4·9
1 : 0	0·1	0·006	11·7	5·5	5·2
Exp. 14.					
2 : 5	0·05	0·036	5·5	4·0	3·9
2 : 1	0·05	0·017	9·9	4·6	4·4
5 : 1	0·05	0·008	11·1	5·0	4·7
1 : 0	0·05	0·003	11·8	5·4	5·0
Exp. 15.					
2 : 5	0·025	0·018	8·1	3·9	3·8
2 : 1	0·025	0·008	10·6	4·6	4·1
5 : 1	0·025	0·004	11·5	4·9	4·3
1 : 0	0·025	0·001	11·8	5·3	4·5

D. *Sodium formate and formic acid.*

Ratio (Salt/Acid)	Concentration of total formate (*M*)	Concentration of CH_2O_2 (*M*)	Corrected rate of fermentation cc. per 5′	*p*H Beginning	*p*H End
Exp. 16.					
5 : 1	0·5	0·083	0	4·1	4·2
15 : 1	0·5	0·031	0·03	4·6	4·6
1 : 0	0·5	0·004	5·9	5·7	5·4
Exp. 17.					
5 : 1	0·2	0·033	0·04	4·1	4·2
15 : 1	0·2	0·012	0·5	4·5	4·7
1 : 0	0·2	0·001	10·6	5·3	4·9
Exp. 18.					
5 : 1	0·1	0·017	0·2	4·1	4·3
15 : 1	0·1	0·006	6·7	4·5	4·5
1 : 0	0·1	0·0007	11·5	5·1	4·7
Exp. 19.					
5 : 1	0·05	0·008	4·3	4·1	4·2
15 : 1	0·05	0·003	8·6	4·4	4·3
1 : 0	0·05	0	11·8	4·9	4·4
Exp. 20.					
5 : 1	0·025	0·004	8·3	4·0	3·8
15 : 1	0·025	0·0015	8·8	4·3	4·0
1 : 0	0·025	0	11·0	4·7	4·1

Table I (*continued*).

E. *Potassium formate and formic acid.*

Exp. 21.

Ratio (Salt/Acid)	Concentration of total formate (*M*)	Concentration of CH_2O_2 (*M*)	Corrected rate of fermentation cc. per 5	p_H Beginning	p_H End
5 : 1	0·5	0·083	0·02	4·2	4·2
15 : 1	0·5	0·031	0·14	4·6	4·6
1 : 0	0·5	0·005	5·6	5·5	5·3
Exp. 22.					
5 : 1	0·2	0·033	0·01	4·2	4·2
15 : 1	0·2	0·012	0·7	4·5	4·6
1 : 0	0·2	0·0009	7·5	5·3	4·9
Exp. 23.					
5 : 1	0·1	0·017	0·5	4·1	4·2
15 : 1	0·1	0·006	5·0	4·5	4·5
1 : 0	0·1	0·0004	7·7	5·1	4·6
Exp. 24.					
5 : 1	0·05	0·008	4·4	4·0	4·1
15 : 1	0·05	0·003	7·3	4·4	4·2
1 : 0	0·05	0	8·2	4·9	4·3
Exp. 25.					
5 : 1	0·025	0·004	6·6	4·0	3·8
15 : 1	0·025	0·0015	7·5	4·3	4·0
1 : 0	0·025	0	7·9	4·6	4·1

F. *Ammonium formate and formic acid.*

Exp. 26.

Ratio (Salt/Acid)	Concentration of total formate (*M*)	Concentration of CH_2O_2 (*M*)	Corrected rate of fermentation cc. per 5′	p_H Beginning	p_H End
5 : 1	0·5	0·083	0·04	4·2	4·2
15 : 1	0·5	0·031	0·04	4·7	4·7
1 : 0	0·5	0·004	4·9	5·4	5·4
Exp. 27.					
5 : 1	0·2	0·033	0·05	4·1	4·2
15 : 1	0·2	0·012	0·8	4·5	4·7
1 : 0	0·2	0·0016	7·3	5·4	4·9
Exp. 28.					
5 : 1	0·1	0·017	0·4	4·1	4·2
15 : 1	0·1	0·006	4·5	4·5	4·4
1 : 0	0·1	0·0009	8·4	5·0	4·5
Exp. 29.					
5 : 1	0·05	0·008	5·1	4·0	4·2
15 : 1	0·05	0·003	9·4	4·4	4·2
1 : 0	0·05	0	11·2	4·7	4·3
Exp. 30.					
5 : 1	0·025	0·004	8·3	4·0	3·9
15 : 1	0·025	0·0015	10·7	4·3	4·0
1 : 0	0·025	0	11·3	4·6	4·0

p_H gradually increases as the concentration of total acetate decreases. In the 0·5 *M* curve, the point at p_H 4·7, at which the ratio of salt : acid = 2 : 1 and the amount of total acid 0·167 *M*, is a typical inflexion point, and the 0·2 *M* curve may also be regarded as having an inflexion point. These curves agree with the so-called "characteristic curve" described by Hägglund and Augustson [1925, 1]. In the 0·1 *M*, 0·05 *M* and 0·025 *M* curves, there is no inflexion point, but these curves can be considered as "characteristic curves," owing to the rapid change in rate of fermentation when the p_H value is slightly changed.

According to Hägglund and Augustson's observations [1925, 1], the optimum p_H value is variable, according to the concentration of the acetate buffer solution. The optimum value moves to the acid side in lower concentrations of the acetate buffer solution. In the present experiments an optimum p_H value was never definitely attained, although in one or two cases it was closely approached. It lies on the alkaline side of the p_H values used.

The concentration of the buffer solution appeared to have a remarkable effect on the rates of fermentation, when these were compared at constant p_H. In discussing this question, however, care should be taken to ascertain as far as possible the independent rôle of each constituent of the buffer solutions, viz. free acid, hydrogen ions, acetate ions, sodium ions, sodium acetate.

Fig. 3 shows the relation between the rate of fermentation and the amount of free acid, and it is seen that the rate is almost independent of the total concentration and of the nature of the cation. As the amount of free acid increases,

the rate of fermentation diminishes rapidly at first until the concentration of about 0·1 *M* is reached; it then falls more slowly, and gradually approaches zero. The rate, as pointed out by Hägglund and Augustson [1925, 1], is seen to be very sensitive to the presence of free acid, a concentration of 0·2 *M* reducing it from about 10–12 cc. to 0·2 cc. per 5 minutes. In short, the fact that the rate of fermentation increases as the concentration of acetate-acetic acid buffer solution decreases is principally due to the variation in the amount of free acid, as found by Johannessohn [1912], Euler and Heintze [1919].

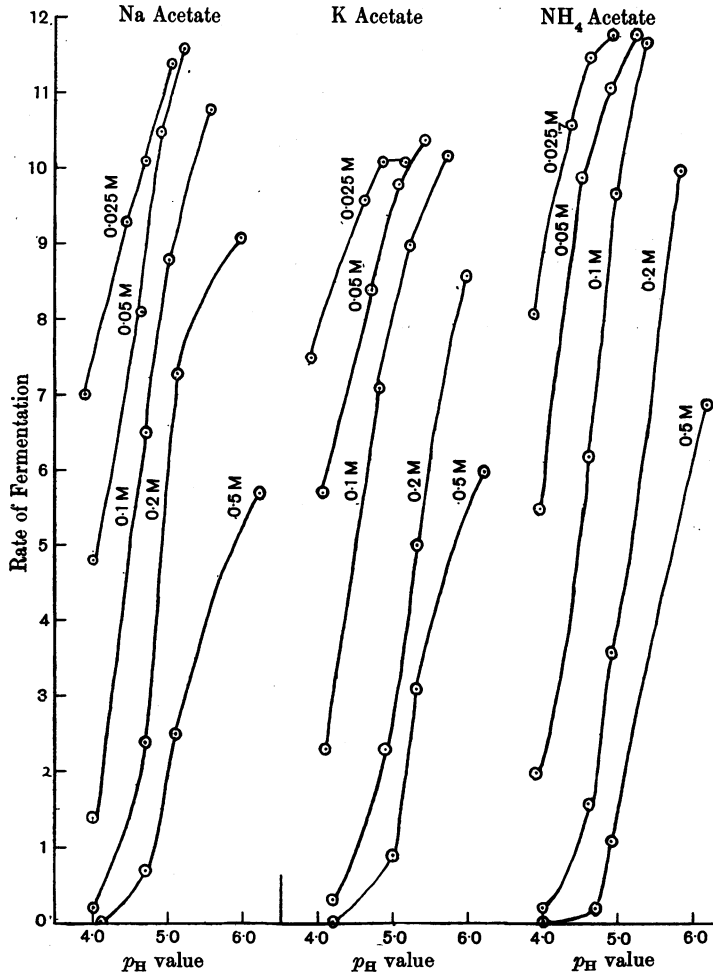

Fig. 1.

This effect is no doubt a complex one, due in part to hydrogen ions and in part to the undissociated molecules of the acid, but the influence of the latter is much greater than that of the former.

Fig. 2, which deals with fermentation in presence of formate, is of much the same character as Fig. 1. It shows that the rate of fermentation with these buffer solutions increases remarkably as their concentration diminishes, and that this does not depend upon the variety of cations ($Na^{\cdot}$, $K^{\cdot}$, and $NH_4^{\cdot}$).

Fig. 4 shows that, as in the case of the acetate-acetic acid buffer solutions, the rate of fermentation with formate-formic acid buffer solutions is controlled by the amount of free acid and is independent of the total concentration.

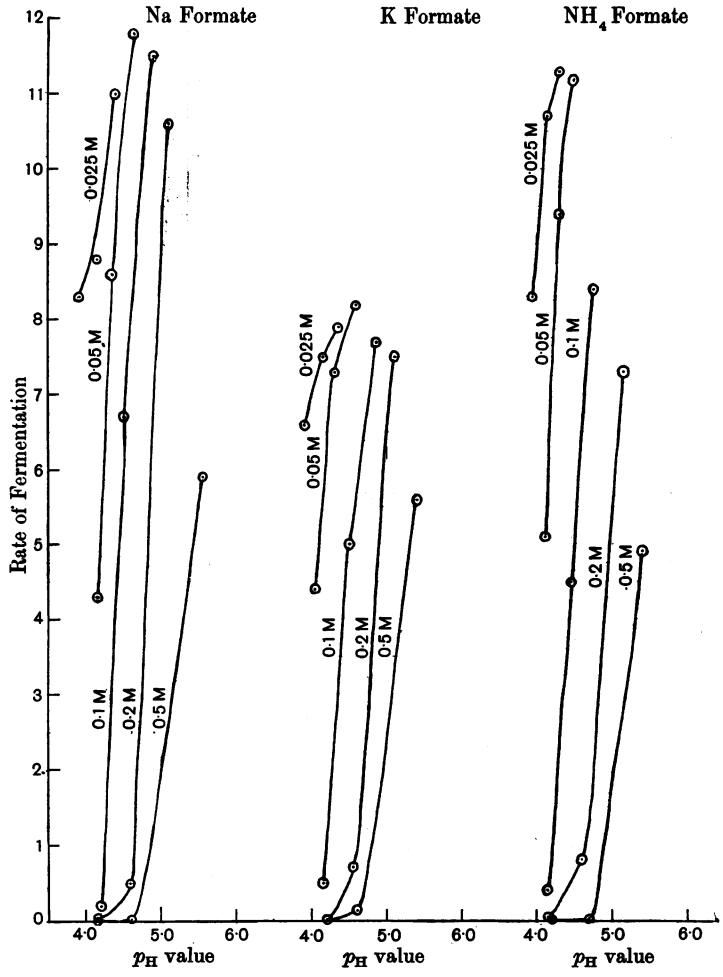

Fig. 2.

The relation between the rate of fermentation and the concentration of acetic and formic acids in the medium is approximately represented by a hyperbolic curve (see Figs. 3 and 4). The observations did not afford any evidence in confirmation of Johannessohn's observation [1912] that formic

acid and its higher homologues accelerate yeast fermentation at a sufficient degree of dilution, but the conditions of the experiment were very different from his.

No specific effect of formates, as compared with acetates, was observed. It was, however, found that the concentration of formic acid required to produce a given rate of fermentation was always much less (5–8 times) than that of the acetic acid required for the same purpose.

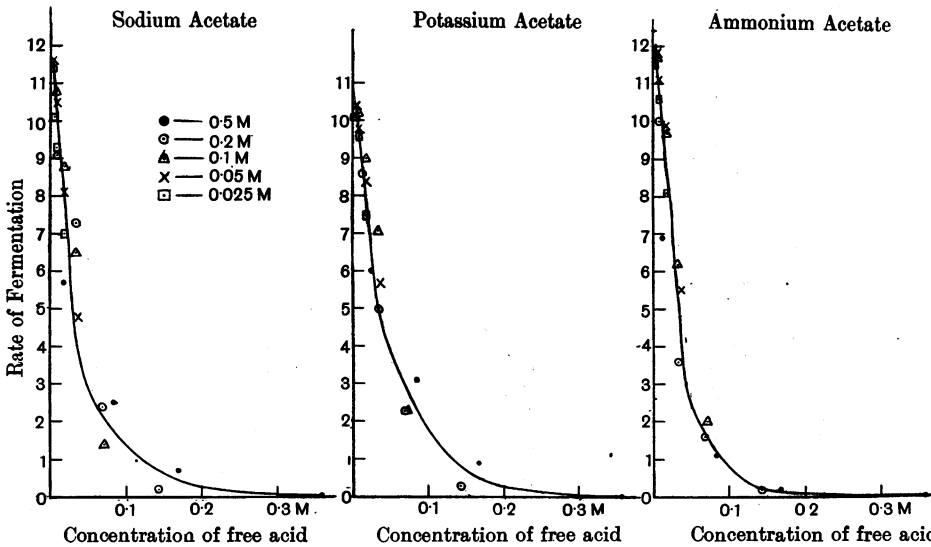

Fig. 3.

No direct comparison is possible of these results with those of Euler and Cassel [1913] and Euler [1919] in which noticeable accelerations were obtained by adding solutions of the formates of Na, K, and NH_4 to mixtures of yeast and sugar solution.

Summary.

(1) Fermentation by living yeast was observed with 0·5 *M*, 0·2 *M*, 0·1 *M*, 0·05 *M* and 0·025 *M* concentrations of acetic acid and acetate buffer solutions, the Na, K and NH_4 salts being used, and with the same concentrations of formic acid and formate buffer solutions.

(2) The rate of fermentation rapidly increased as the total amount of acid decreased, in both cases.

(3) It is suggested that a hyperbolic relation exists between the rate of fermentation and the amount of free acid in both these buffer solutions.

(4) At constant concentration of acid the rate is almost independent of the total acetate or formate concentrations.

(5) When the rate of fermentation is plotted against the p_H of the medium, the total concentration of acetate or formate (*i.e.* acid + salt) being kept constant, the form of the curve is the same for the cations, $Na^{\cdot}$, $K^{\cdot}$, and $NH_4^{\cdot}$.

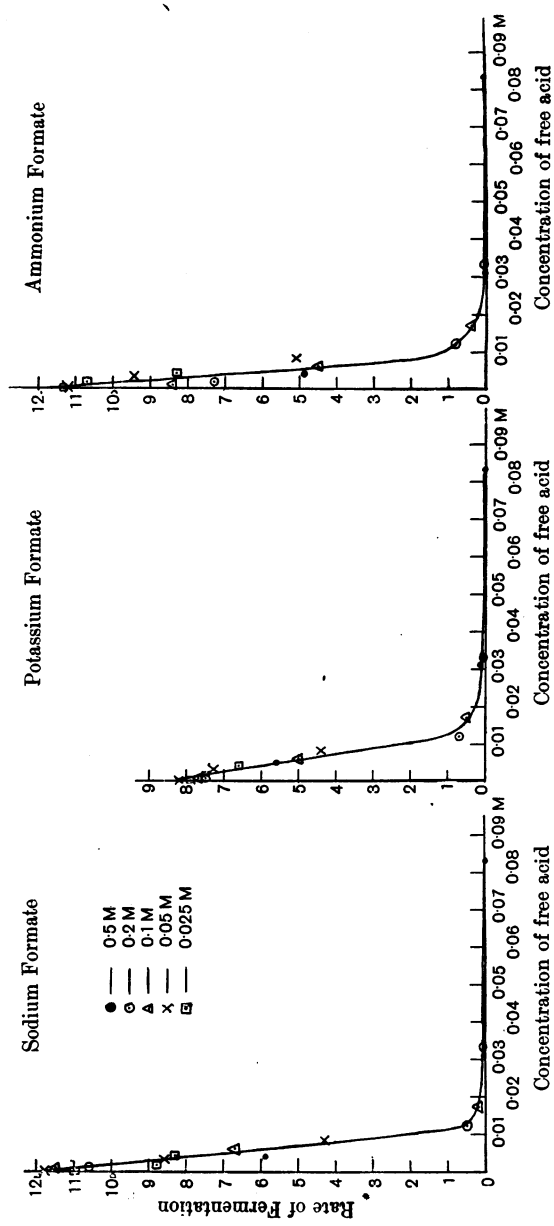

Fig. 4.

(6) No specific effect of formates as compared with acetates was observed, but formic acid is 5–8 times as potent as acetic acid in diminishing the rate of fermentation.

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