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THE INFLUENCE OF ACIDS ON THE RESPIRATION OF SACCHAROMYCES CEREVISIAE

THE [illegible] OF ACIDS ON THE RESPIRATION OF [illegible]

by

[illegible]

A THESIS

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INTRODUCTION

Certain conditions of the substrate which govern the physiological activities of microorganisms such as temperature, oxygen tension, osmotic pressure, and acid content are matters of fundamental interest to the bacteriologist.

Many studies have been made concerning the physiological action of acids. As a result several suggestions have been made regarding their mode of action. None of these are completely satisfactory. The order of toxicity of different acids upon various forms of life is often reversed. A quantitative relationship between various acids and their effect upon the same organism has never been induced. The failure may be due in part to the fact that in the majority of cases a comparison has been made of their activities at a single concentration of acid. Also, in many instances the nature of the experiments made a precise determination of the degree of toxicity impossible. If the comparative toxicity of several acids at several concentrations could be determined very accurately, the results might be expected to provide a better insight regarding the factors involved.

The method used in the present work was based upon the effect of acids in depressing the rate of oxygen uptake of yeast as measured by the Warburg apparatus. Preliminary experiments indicated that this method was sensitive to very small differences in acidity and that measurements over a wide range of concentrations could be satisfactorily reproduced. Using this method a study was made of the activity of fourteen acids. With acetic acid an attempt was made to determine the comparative toxicity of the undissociated acid, the acetate ion and the hydrogen ion.

LITERATURE REVIEW

The literature dealing with the biological significance of acids is vast due largely to the diversity of experimental material which has been studied. Representative of the biological branches are the experiments on the feeding of acids to dogs by Walter (1877), intravenous injection of acids by Smith (1909), the effect upon fish and tadpoles when acids were added to the external medium by Loeb and Wasteneys (1911), the effect of acids upon the ciliate infusoria by Chilett (1919) and (1921), upon skeletal muscle by Dale and Mines (1911), upon the swelling of gelatin by Fischer and Hooker (1918), upon the oxidation of oxalic acid by hydrogen peroxide, Katcher (1923), upon plant root-hairs by Kahlenberg and True (1896) and Ewald (1896), upon worms by Forster (1916), and upon the activation of starfish eggs by acids, Lillie (1906-27).

In the field of microbiology among the early workers are Pasal and Krenig (1897), Pasal and Krauz (1910), Beel (1897) and (1902), Kitasato (1888), Winslow and Lochridge (1906), Rosenbrand and Rosenblatt (1909) and Prue (1908). More recent workers include Koch (1940), Tetsumoto (1940), Lawrence (1940), Fabian and Wadsworth (1939), Shaheimer and Fabian (1940), Levine and Fellers (1940), Williams and Levine (1943), Erickson and Fabian (1941), McCalla (1940), Lwoff and Morell (1942) and Burgois and Dorablect (1943).

It is to be expected that with such wide choices of material and variations in experimental procedures there has been a marked lack of agreement in the results. Generally speaking, with those organisms affected by very small concentrations of acid the strong inorganic acids

are more toxic than the weaker organic acids. The reverse is usually true of those forms of life which are appreciably affected only by greater amounts of acid. Representative of the forms susceptible to low acid concentration are animal tissue, plant tissue and bacteria. Yeasts and molds represent a class of life relatively much more resistant.

Practically all workers have recognized that some factor other than H-ion concentration is responsible for the physiological action of acids. Paul and Krouk (1896, working with bacteria pointed out that although strong acids acted generally in relation to their H-ions there was also a specific action of the particular anion or undissociated acid. Kahlenburg (1898, studying the action of acid upon the sense of taste found that acetic acid had a sour taste about four times as great as could be explained on the basis of H-ion concentration. Winslow and Lochridge (1908) observed that with hydrochloric and sulphuric acids the toxicity toward bacteria was due to H-ion. However, they found the weak acids, acetic and benzoic, to be toxic at concentrations at which they were only slightly dissociated. They concluded that the effect of the weak acids was due to the whole molecule. Paul and Rona (1910) considered that with bacteria the toxic action of the H-ions of weak organic acids was catalysed by anions. Wolf and Shunk (1921, studying the tolerance to acids of certain plant pathogens found that some factor besides the concentration of H-ions was operating to inhibit the growth of their cultures. Jones (1922) found that in their toxicity for leucocytes lactic, acetic and butyric acids had a specific action in addition to that of the dissociated H-ions. Cooper and Biggs (1926) studying the biological significance of cis-trans isomerism found

that germicidal action of the cis-trans isomers, maleic and fumaric acids was not determined by the H-ion concentration but must depend upon the stereochemical configuration of the molecule. Taylor (1916) found that in the treatment of wounds infected with <u>Bacillus [illegible]</u> acetic acid was much more effective than the strong acids, hydrochloric and nitric.

Accepting that the toxicity of weak acids was probably due in part to the action of the undissociated acid molecules a few workers have [illegible] proportions of H-ion, anion and undissociated acid molecule were altered by the addition of strong acids and salts with a common ion. Collett (1919), studying the toxicity of acids to ciliate infusoria found that [illegible] of organic acids and hydrochloric acid [illegible] toxicity was progressively diminished with the addition of [illegible] hydrochloric acid. [illegible] the addition of hydrochloric acid decreased the concentration of the [illegible] anion she believed [illegible] suggested that the [illegible] anions were toxic. In a later [illegible] Collett (1921) found [illegible] of formic, acetic, butyric, and valeric acids with [illegible] their salts were more toxic [illegible] would [illegible] of the mixtures. She felt [illegible] results [illegible] were [illegible], but lactic, malic, [illegible] tartaric [illegible] found no evidence that the anion or undissociated acid molecule was toxic at the concentrations used.

Lillie (1926-27), studying the activation of starfish eggs by acids added their sodium salts to solutions of organic acids. This caused a decrease in the H-ion concentration but the rate of activation increased by approximately 10 per cent. Lillie concluded that apparently only the undissociated molecules penetrated the egg freely. He assumed that after

having penetrated the acid dissociated in the interior of the egg furnishing the H-ions which effected activation.

Katagiri (1925), working with buffer solutions of Na, K and NH4 and salts of formic and acetic acids found that the concentration of buffer solution had a marked effect on the fermentation of sucrose by yeast in solutions of the same pH. He also determined that at a constant concentration of acid the rate of fermentation was almost independent of the total acetate or formate concentration and, therefore, independent of pH.

Root (1925), found that the addition of sodium citrate markedly increased the toxicity of citric acid toward Bacillus coli communis.

On the comparative toxicity of the homologues of the saturated fatty acid series there seems to be nearly complete agreement. With the exception of the first member toxicity increases with molecular weight. However, Bell (1917), found that with a group of pathogenic bacteria and Escherichia coli the bactericidal action of the fatty acids decreased as the molecular weight increased. For germicidal action he found the reverse to be true.

Tetsumoto (1934), found that the toxicity of the halogenated saturated and unsaturated fatty acids was greater than that of the parent acids.

The relative toxicity of the various acids is one of the few things which can be used as a basis of comparing the results on different organisms. Following is a tabulation taken from the literature showing the author, experimental material and order of toxicity of a few acids on the basis of normality.

Author	Material	Order of Toxicity
Winslow and Lockridge (1906)	Bacteria	HCl > H_2SO_4 > acetic
Pums (1908)	"	acetic > lactic > HCl
Wyeth (1918)	"	HCl = lactic > acetic
Yant (1925)	"	acetic > lactic > HCl = H_2SO_4
Reid (1932)	"	lactic > acetic
Hunheimer and Fabian (1940)	"	HCl > lactic > acetic
Shillinglaw and Levine (1943)	"	lactic > acetic
Kahlenburg and True (1896)	Plant Seedlings	HCl = H_2SO_4 > lactic > acetic
Dial (1900)	Yeasts	HCl > acetic
Rosenbrand and Rosenblatt (1904)	"	HCl = H_2SO_4 > acetic > lactic
Taylor (1923)	"	HCl > H_2SO_4 > acetic > lactic
Robins and Wadsworth (1930)	"	acetic > lactic

The above data indicate that, of the inorganic acids, hydrochloric, the stronger acid, is more toxic than sulfuric. Comparing lactic and acetic acids it is generally true that lactic, the more highly dissociated acid, is more toxic than acetic toward bacteria but that the reverse is true for yeasts.

EXPERIMENTAL

Experiments were carried out to determine the effect upon the rate of oxygen up-take of the yeast, Saccharomyces cerevisiae, by the following factors:

1. Several inorganic and organic acids alone and in 0.01 molar d-glucose solutions
2. Sodium hydroxide alone and in 0.01 molar d-glucose solution
3. Sodium salts of several acids in 0.01 molar d-glucose solutions
4. Combinations of inorganic acids with acetic acid in 0.01 molar d-glucose solutions
5. Combinations of the sodium salts of inorganic acids with acetic acid and combinations of sodium acetate with acetic acid in 0.01 molar d-glucose solutions

Method of determining oxygen up-take

All of this work reported here was done with the Warburg apparatus, Dixon (13-1). Briefly this apparatus consists of a cup having a capacity of about 20 ml. connected by means of a ground glass joint to a delicate manometer. The cup is provided with a small receptacle in which strong potassium hydroxide solution may be placed to absorb the carbon dioxide evolved during the respiration of the yeast.

The factors which effect the pressure on the manometer are temperature, barometric pressure, vapor pressure and gases evolved or

taken up by the yeast culture. The temperature and barometric pressure are determined by a cup holding only distilled water and a run is made on distilled water each time that a sample is run. The vapor pressure is considered constant and is included with a cell constant. With the substances employed no gases other than carbon dioxide are given off during respiration and thus the amount of oxygen taken up can be calculated by multiplying the manometer pressure by a constant.

Preparation of yeast culture

The culture was prepared in the following way: Roux flasks were inoculated with a suspension of the growth from an agar slant and incubated for three days at room temperature. This growth was washed off, centrifuged, the supernatant decanted, sterile distilled water added, and the suspension completely mixed again. This procedure was repeated until the culture had been washed four times with sterile distilled water. It was taken up in more sterile distilled water approximating 10 ml. for each Roux flask and aerated for one-half hour. The total solids were determined and the suspension adjusted so as to contain the desired concentration of yeast solids. This concentration was such that the solutions pipetted in the Warburg cups contained 2.5 mg. of yeast solids or less. For part of the experimental work four milliliters were used per cup and during another part two milliliters.

All determinations were made in duplicate and in some cases determinations were repeated many more times in order to test the reproducibility of results. It was found that suspensions were unchanged after two weeks but a new suspension was made each week. Each suspension was checked six times for activity before use and at least once each day thereafter.

The amount of oxygen up-take was determined at 15 minute intervals over a period of one hour. The temperature in all cases was kept constant at 30° C. with a maximum variation of 0.2 degree. In all cases except where the acids or salts were being studied alone, the solutions were 0.01 molar in d-glucose. It was determined that concentrations of d-glucose from 0.005-0.05 molar had the same effect on oxygen up-take.

The reason for using d-glucose solutions was two-fold. The yeast was able to oxidize many of the organic acids and their salts. In fact, at low concentrations of acetic acid the rate of oxygen up-take was nearly the same as with 0.01 molar d-glucose. On the other hand, none of the inorganic acids could be oxidized by the yeast. In order to compare two acids such as acetic and hydrochloric, it was necessary to have present a readily oxidizable compound such as d-glucose. The second reason for including d-glucose in the solutions was to reduce the experimental error incident to determining the amount of oxygen taken up. In the presence of d-glucose the oxygen up-take is approximately 10 times that in the absence of d-glucose.

Standardization of yeast suspension

It was found early in the work that the milligrams of oxygen taken up per milligram of dry yeast cells was not a satisfactory basis of standardization due to the fact that two suspensions made in apparently an identical manner did not show the same amount of oxygen up-take. When the oxygen up-take was determined for several concentrations of an acid in the presence of 0.01 molar d-glucose using first one suspension and then another the values did not agree. If the value obtained for 0.01 molar d-glucose solution was taken as 100 and the values in the presence of the various amounts of acid calculated as a percentage of the amount of oxygen

up-take in the absence of acid the results using different suspensions agreed very well. Therefore, the basis finally adopted was the rate of the oxygen up-take from 0.01 molar d-glucose solution and all results throughout this work are calculated in such a manner that they represent per cent of the oxygen up-take of a solution of 0.01 molar d-glucose. The reagents used were all J. T. Baker's analysed chemicals or Pfanstiel analysed chemicals with the exception of a few organic acids not available in that quality.

EXPERIMENTAL RESULTS

Experiments were conducted to determine the influence of several acids on the rate of oxygen up-take by Saccharomyces cerevisiae over a certain range of concentrations. The concentrations were chosen so as to determine the rate of oxygen up-take over the range which caused a very slight inhibition to almost complete inhibition. It was found that a determination of the amount of acid completely to inhibit the oxygen up-take was not exactly reproducible. This is in accord with many studies in bacteriology such as on the action of heat or toxic substances wherein it has been found that a 99 per cent reduction in the numbers of bacteria is a more reliable index for comparing the germicidal effect than is 100 per cent reduction. There is the additional factor in this work that the determination of one or two cubic millimeters is attended by the same errors in reading as are the determination of 100 or 200 cubic millimeters.

Reproducibility of results

The reproducibility of results using different suspensions of the yeast with solutions of identical content are shown below. Data are shown for several concentrations of four acids and a total of six different suspensions.

Equivalents per liter	Oxygen up-take			
Suspension No.	15	16	22	23
Acetic acid				
.005	88.5	87.2	89.8	88.0
.01	71.4	69.0	70.1	73.0
.02	53.0	50.7	49.0	49.3
.04	24.0	-	22.6	22.6
.08	-	7.4	-	4.1

Equivalents per liter	Oxygen up-take		
Suspension No.	23	23	
Hydrochloric acid			
.01	80.7	80.2	
.02	72.4	70.7	
.04	52.0	49.0	
.08	-	38.6	
Suspension No.	22	23	24
Sulfuric acid			
.02	78.4	75.8	71.9
.04	-	67.6	58.2
.08	40.1	45.0	54.8
Suspension No.	18	22	
Lactic acid			
.05	86.1	91.0	
.10	82.1	86.5	
.20	64.8	75.0	
.50	14.1	16.5	

Each of the above values is the average of two determinations. The deviation between duplicates runs about the same as when different suspensions are used in solutions of identical content.

As the data show the precision of the results is not as good as with ordinary chemical determinations but is compare favorably with the usual bacteriological quantitative work such as plating. The experiments on the influence on the rate of oxygen up-take of Saccharomyces cerevisiae by acids were carried out first with acid solutions alone and then with acid solutions containing 0.01 molar d-glucose.

Tables (1-15) show the results in some detail. For each concentration of acid the pH at the beginning and at the end of one hour's run are shown. The oxygen up-take as a percentage of that of 0.01 molar d-glucose are given for each 15 minute interval and for the total of one hour. Data are not included for 15 minute intervals on some of the acids in the presence of d-glucose for the amount of oxygen up-take was too small to permit a valid comparison of the amounts taken up in 15 minute intervals. In Figs. (1-6, the oxygen up-take during the one hour period, expressed as a percentage of the oxygen up-take of 0.01 molar d-glucose is plotted against the concentration of the acid expressed as equivalents per liter.

Table 1. Influence of sulfuric acid on oxygen up-take of [illegible]

A. Sulfuric acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take: 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	[illegible]	-					[illegible]
.01	[illegible]	[illegible]					7.8
.02	1.90	[illegible]					[illegible]
.04	[illegible]	1.80					[illegible]
.08	[illegible]	[illegible]					[illegible]
.1	[illegible]	[illegible]					[illegible]
.1	[illegible]	[illegible]					[illegible]
.2	[illegible]	[illegible]					[illegible]
.24	[illegible]	[illegible]					[illegible]
.28	[illegible]	-					[illegible]
.3	[illegible]	[illegible]					[illegible]
.4	[illegible]	[illegible]					1.6

B. Sulfuric acid in 0.01 molar bicarbonate solution

0	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	100
.005	[illegible]	[illegible]	13.0	[illegible]	[illegible]	[illegible]	[illegible]
.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.02	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.04	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.06	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.08	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.1	[illegible]	[illegible]	14.1	[illegible]	[illegible]	[illegible]	[illegible]
.12	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.15	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 7. Influence of hydrochloric acid on oxygen up-take of [illegible] [illegible]

A. Hydrochloric acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0							6.7
.004							10.5
.01							12.0
.02							12.2
.04							12.7
.06							13.8
.08							14.[illegible]
.10							13.5
.12							11.8
.14							[illegible].9
.16							[illegible].7
.18							[illegible].[illegible]
.20							[illegible].[illegible]
.22							1.7

B. Hydrochloric acid in 0.01 molar [illegible] solution

0	.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	100
.004	[illegible].91	[illegible].96	[illegible]0.1	[illegible].0	[illegible].8	[illegible]0.1	86.1
.01	[illegible].11	[illegible].00	19.2	[illegible]0.[illegible]	14.[illegible]	13.1	[illegible]7.9
.02	1.40	1.[illegible]2	17.[illegible]	17.0	15.1	14.9	66.2
.04	1.50	1.4[illegible]	15.[illegible]	13.[illegible]	11.1	10.[illegible]	52.7
.08	1.21	1.[illegible]0	10.[illegible]	10.0	8.[illegible]	6.7	35.6
.12	1.08	1.08	6.1	.1	[illegible].6	[illegible].[illegible]	19.5

Table 3. Influence of nitric acid on oxygen up-take of [illegible]

A. Nitric acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	-	-					10.8
.001	[illegible]	-					9.6
.005	[illegible]	-					11.6
.01	[illegible]	-					12.8
[illegible]	[illegible]	-					14.3
.04	1.50	1.45					10.8
.08	[illegible]	1.18					2.5
.1	[illegible]	1.00					0

B. Nitric acid in 0.01 molar glucose solution

0	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	100
.005	[illegible]	-	19.4	[illegible]	[illegible]	22.8	[illegible]
.01	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.03	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.04	-	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.04	1.90	1.[illegible]	12.[illegible]	17.[illegible]	13.0	10.[illegible]	[illegible]
.06	1.90	1.90	7.8	8.[illegible]	[illegible]	[illegible]	[illegible]
.08	1.[illegible]	1.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	16.1
.10	1.15	1.14	1.5	0.7	0.9	0.8	[illegible]

Table 4. Influence of sodium hydroxide on oxygen up-take of [illegible]

A. Sodium hydroxide

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0							15.6
.001							17.3
.00[illegible]							16.9
.00[illegible]							14.8
.01							15.[illegible]
.02							14.4
.04							15.5
.06							0

B. Sodium hydroxide in 0.01 molar [illegible] solution

0	4.29	-	[illegible].8	[illegible].1	[illegible].8	[illegible].4	100
.001	5.98	[illegible]	[illegible].2	[illegible].6	[illegible].[illegible]	[illegible].5	110
.00[illegible]	10.[illegible]	[illegible]	[illegible].7	[illegible].[illegible]	11.3	15.4	[illegible].[illegible]
.01	11.0[illegible]	[illegible]	[illegible].[illegible]	[illegible].0	[illegible].7	[illegible].[illegible]	[illegible].4
.02	11.40	11.[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].5
.04	11.50	-	[illegible].7	[illegible].7	[illegible].1	[illegible].1	[illegible].5
.06	11.[illegible]0	11.30	2.1	[illegible].[illegible]	[illegible].[illegible]	[illegible].1	10.9

Table 5. Influence of formic acid on oxygen up-take of Saccharomyces cerevisiae

A. Formic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	5.20	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	10.0
.0005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	29.4
.001	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	30.8
.002	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	50.7
.003	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	59.4
.004	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	36.2
.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

B. Formic acid in 0.01 molar d-glucose solution

0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	100
.0005	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.001	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.002	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.003	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.004	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 6. Influence of acetic acid on oxygen up-take of [illegible]

A. Acetic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	5.20	-	2.1	1.9	2.5	1.8	7.5
.005	3.47	4.13	22.5	23.6	26.1	26.5	101
.01	3.35	3.50	21.4	22.1	24.2	25.0	92.7
.02	3.22	3.30	15.2	15.9	19.3	20.0	71.5
.03	3.13	3.20	8.9	11.0	11.9	12.6	44.7
.04	3.07	3.10	4.3	7.0	5.5	6.6	24.4
.06	2.97	3.02	1.8	2.9	3.0	3.7	11.4
.08	2.96	-	1.1	1.3	1.3	2.3	6.3

B. Acetic acid in 0.01 molar d-glucose solution

0	4.29	-	22.8	24.6	25.8	27.4	100
.005	3.47	3.44	18.8	21.0	23.2	24.3	87.2
.01	3.35	3.40	14.7	16.1	18.0	18.2	69.0
.02	3.18	3.30	10.7	12.2	13.7	14.1	50.7
.03	3.14	3.08	5.2	8.1	8.4	9.6	31.5
.06	2.98	3.04	1.7	1.7	2.4	3.5	9.3
.08	2.93	2.95	1.4	1.7	2.1	2.2	7.4
.10	2.89	2.90	1.0	1.4	1.4	1.5	5.3
.12	2.83	2.85	0.7	0.8	0.9	1.1	3.5

Table 7. Influence of propionic acid on oxygen [illegible] of Saccharomyces cerevisiae

A. Propionic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take: 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.001	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.002	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.003	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.004	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.006	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.007	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.008	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.009	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.010	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

B. Propionic acid in [illegible] per liter [illegible]

0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	100
.001	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.002	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.003	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.004	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.02	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.03	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.04	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.06	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.08	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 8. Influence of glycolic acid on oxygen uptake of Saccharomyces cerevisiae

A. Glycolic acid

Equivalents per liter	pH at start	pH at end	Oxygen uptake: 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	[illegible]	[illegible]					10.0
.01	[illegible]	-					15.0
.02	[illegible]	[illegible]					13.4
.03	[illegible]	[illegible]					[illegible]
.04	[illegible]	[illegible]					[illegible]
.05	[illegible]	-					16.1
.06	[illegible]	[illegible]					11.1
.07	[illegible]	[illegible]					6.7
.08	[illegible]	[illegible]					4.9
.10	[illegible]	[illegible]					[illegible]
.12	[illegible]	[illegible]					3.7

B. [illegible] acid to .01 molar [illegible]

0	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	100
.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.01	[illegible]	[illegible]	[illegible]	1.9	1.9	[illegible]	86.2
.02	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	19.8	80.4
.04	[illegible]	[illegible]	11.9	[illegible]	11.[illegible]	[illegible]	[illegible]
.06	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	19.9
.08	[illegible]	[illegible]	0.4	0.3	0.4	0.4	1.5

Table 3. Influence of lactic acid on oxygen up-take of Saccharomyces cerevisiae

A. Lactic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 1 minute periods 2nd	3rd	4th	Total over 1 hr. period
0			1.4	1.3	1.6	1.0	5.3
.01			11.4	1 .1	13.8	13.7	51.0
.0			11.7	1 .4	13.7	14.5	53.0
.0			13.4	13.7	1 .8	1 .2	53.6
.10			15.	15.	16.5	1 .7	66.8
.1			1 .1	17.7	1 .7	17.0	6 .5
.20			13.1	1 .4	1 .7	16.	60.
.30			11.1	1 .	13.4	14.	52.2
.40			.8	4.0	4.8	10.1	36.3
.50			3.1	3.1	3.	3.4	12.8
.60			1.6	1.	1.4	1.1	5.6
.70			0.	0	0.6	0.5	1.8

B. Lactic acid in 0.01 molar Sorensen solution

0	.1	-	1.4	1.9	.7	1.6	100
.05	.1	.11	3.1	2.3	3.	2.1	11.1
.0	.7	.68	1.3	1.4	1.6	2.9	85.1
.10	.9	.9	20.8	1 .4	20.4	21.0	82.4
.1	.37	.30	17.7	18.1	18.1	18.4	72.3
.20	.21	.23	17.0	17.4	17.	17.8	69.8
.25	.15	.18	14.1	16.	17.4	17.5	65.3
.50	.00	.0	4.7	3.8	3.4	3.4	14.3
.75	1.90	-	0	0	0	0	0

Table 10. Influence of succinic acid on oxygen up-take of Saccharomyces cerevisiae

A. Succinic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	4.32	4.85	4.0	4.2	3.8	4.0	15.9
.005	3.80	3.[illegible]	3.9	4.0	3.5	3.4	14.8
.01	3.90	3.81	4.0	3.8	3.4	3.4	14.7
.02	3.10	3.14	[illegible]	4.1	3.6	3.5	15.6
.04	[illegible]	[illegible]	5.2	4.7	4.5	5.0	19.5
.08	[illegible]	[illegible]	[illegible]	4.8	[illegible]	4.8	20.0
.16	[illegible]	[illegible]	6.8	[illegible]	[illegible]	6.1	[illegible]
.32	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.70	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

B. Succinic acid in 0.01 molar Glucose

0	4.17	-	[illegible]	[illegible]	[illegible]	[illegible]	100
.005	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.01	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]		[illegible]		[illegible]	[illegible]	[illegible]
.04	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	87.0
.08	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	13.3	81.4
.16	[illegible]	1.60	20.0	[illegible]	16.7	16.7	77.5
.32	[illegible]	-	17.7	[illegible]	[illegible]	17.2	69.2
.70	[illegible]	-	14.2	14.0	13.7	13.4	55.3

Table 11. Influence of l-malic acid on oxygen up-take of Saccharomyces cerevisiae

A. l-malic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	[illegible]	[illegible]	[illegible]	[illegible]	3.8	[illegible]	15.9
.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	16.5
.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.02	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	17.0
.04	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.08	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.12	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.16	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.20	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.70	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	17.3
1.00	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.20	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	10.3
1.40	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

B. l-malic acid in 0.01 molar [illegible] solution

0	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	100
.005	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.01	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.02	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.04	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	86.5
.08	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.16	[illegible]	-	19.4	[illegible]	[illegible]	[illegible]	[illegible]
.30	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	72.0
.70	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.00	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.40	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 12. Influence of tartaric acid on oxygen up-take of [redacted]

A. Tartaric acid (41,

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	5.00	[illegible]	[illegible]	[illegible]	2.7	[illegible]	10.0
.01	2.70	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	11.2
.02	[illegible]	[illegible]	[illegible]	[illegible]	3.6	3.5	13.0
.04	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5.2	19.6
.08	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	18.3
.16	1.93	1.92	[illegible]	[illegible]	[illegible]	[illegible]	17.8
.30	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	15.2
.70	1.67	1.71	[illegible]	[illegible]	2.0	1.7	9.9
1.00	[illegible]	1.42	[illegible]	[illegible]	1.9	1.8	8.1
1.40	1.43	[illegible]	[illegible]	[illegible]	1.6	1.6	8.0
1.80	[illegible]	[illegible]	1.4	[illegible]	1.4	1.2	5.7
2.10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.1	5.4

B. Tartaric acid in 0.01 molar d-glucose solution

0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	25.7	100
.01	[illegible]	[illegible]	18.5	[illegible]	[illegible]	[illegible]	83.6
.02	[illegible]	[illegible]	19.2	[illegible]	[illegible]	[illegible]	81.1
.04	[illegible]	[illegible]	16.7	[illegible]	[illegible]	19.4	79.6
.08	[illegible]	[illegible]	18.1	[illegible]	17.4	17.8	[illegible]
.16	1.90	1.98	15.3	15.4	13.[illegible]	11.8	57.9
.30	1.74	1.81	16.4	14.9	[illegible]	7.6	[illegible]
.70	1.55	1.60	12.5	10.[illegible]	[illegible]	6.6	33.9
1.00	1.46	1.50	10.[illegible]	[illegible]	[illegible]	[illegible]	30.7
1.40	1.35	1.41	9.4	[illegible]	[illegible]	[illegible]	26.9
1.80	1.33	1.39	[illegible]	[illegible]	[illegible]	[illegible]	21.5
2.10	1.30	1.31	[illegible]	[illegible]	[illegible]	[illegible]	19.6

Table 13. Influence of pyruvic acid on oxygen up-take of Saccharomyces cerevisiae

A. Pyruvic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	[illegible]	[illegible]	[illegible]	[illegible]	3.1	[illegible]	12.3
.005	2.74	[illegible]	[illegible]	18.2	[illegible]	[illegible]	57.1
.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	87.5
.02	[illegible]	[illegible]	[illegible]	[illegible]	18.7	19.1	79.0
.04	[illegible]	[illegible]	15.4	[illegible]	[illegible]	[illegible]	[illegible]
.08	1.91	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.16	1.69	[illegible]	[illegible]	[illegible]	[illegible]	0.7	[illegible]

B. Pyruvic acid in 0.01 molar d-glucose solution

0	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	100
.005	[illegible]	[illegible] 2.81	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.01	[illegible]	[illegible]	18.9	18.7	[illegible]	[illegible]	[illegible]
.02	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	61.1
.04	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.08	1.82	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.16	[illegible]	[illegible]	[illegible]	[illegible]	1.8	0.7	[illegible]

Table 14. Influence of levulinic acid on oxygen up-take of Saccharomyces cerevisiae

A. Levulinic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take in 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0			2.1	2.5	3.0	3.0	10.5
.005			4.5	4.9	3.9	3.7	16.8
.01			7.8	5.8	4.7	4.8	23.1
.02			9.0	9.1	7.9	7.1	33.0
.03			6.5	6.3	7.0	6.5	26.3
.04			4.5	4.9	4.9	5.0	19.3
.05			4.4	4.5	3.5	3.6	15.5
.10			0.4	0.3	1.3	1.4	4.4
.15			0	0	0	0	0

B. Levulinic acid in 0.01 molar d-glucose solution

0	4.17	-	24.4	23.8	24.3	23.6	100
.005	3.50	3.40	21.3	21.5	21.4	23.1	87.2
.01	3.35	3.31	18.3	18.8	19.0	19.3	75.3
.02	3.15	3.18	11.8	10.3	11.0	11.2	44.9
.04	3.04	3.03	4.3	4.1	4.9	4.8	18.9
.06	2.92	2.97	2.1	2.2	2.1	2.1	8.5
.08	2.85	2.94	1.2	1.1	1.0	1.4	4.7

Table 15. Influence of malic acid on oxygen up-take of [illegible]

A. Malic acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute period 1st	2nd	3rd	4th	Total over 1 hr. period
0	4.97	4.90	3.0	3.2	3.1	3.2	12.3
.00[illegible]	2.66	2.70	3.5	4.0	3.9	2.9	13.4
.01	2.40	2.42	4.3	4.7	4.4	4.2	17.9
.02	2.13	2.1[illegible]	9.0	[illegible]	[illegible].8	4.9	[illegible].3
.04	1.97	1.93	10.[illegible]	[illegible].0	[illegible].9	7.1	[illegible].[illegible]
.08	1.[illegible]6	1.74	9.4	[illegible].7	[illegible].[illegible]	7.8	[illegible].1
.16	1.47	1.[illegible]7	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	8.2	[illegible].[illegible]
.32	1.52	1.[illegible]7	[illegible].4	4.7	[illegible].7	[illegible].6	16.3
.48	1.[illegible]4	-	1.[illegible]	1.[illegible]	[illegible].[illegible]	0	3.7
.50	1.1[illegible]	-	0	0	0	0	0

B. Malic acid in 0.01 molar [illegible]-glucose solution

0	[illegible].[illegible]	-	[illegible].9	[illegible].0	[illegible].[illegible]	[illegible].[illegible]	100
.00[illegible]	[illegible].67	[illegible].7[illegible]	19.8	[illegible].0	[illegible].4	[illegible].6	83.5
.01	[illegible].40	2.42	17.4	19.[illegible]	[illegible].1	19.7	77.5
.0[illegible]	[illegible].1[illegible]	[illegible].[illegible]0	17.[illegible]	18.[illegible]	[illegible].7	17.[illegible]	71.0
.0[illegible]	1.[illegible]5	1.[illegible]4	16.0	[illegible].[illegible]	[illegible].1	14.7	[illegible].2
.08	1.72	1.7[illegible]	13.[illegible]	[illegible].0	[illegible].0	11.0	[illegible].[illegible]
.16	1.53	1.[illegible]4	10.4	[illegible].1	8.[illegible]	[illegible].[illegible]	[illegible].7
.32	1.[illegible]8	1.[illegible]4	[illegible].9	[illegible].[illegible]	4.4	3.7	20.1
.48	1.20	-	4.0	[illegible].9	[illegible].[illegible]	1.2	9.6
.50	1.1[illegible]	-	1.[illegible]	1.[illegible]	.[illegible]	.6	4.2
.60	1.0[illegible]	-	.2	.2	.[illegible]	.2	1.0
.70	1.12	1.17	0	0	0	0	0

Table 16. Influence of fumaric acid on oxygen up-take of [redacted]

A. Fumaric acid

Equivalents per liter	pH at start	pH at end	Oxygen up-take 15 minute periods 1st	2nd	3rd	4th	Total over 1 hr. period
0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2.7	10.0
.005	[illegible]	2.96	[illegible]	[illegible]	[illegible]	2.9	11.2
.01	[illegible]	2.73	[illegible]	[illegible]	[illegible]	[illegible]	18.3
.02	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	6.8	27.1
.04	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	31.8
.06	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	33.7
.07	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

B. Fumaric acid in 0.01 molar d-glucose solution

0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	25.7	100
.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	89.5
.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	85.0
.02	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	86.6
.04	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	77.1
.06	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
.07	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

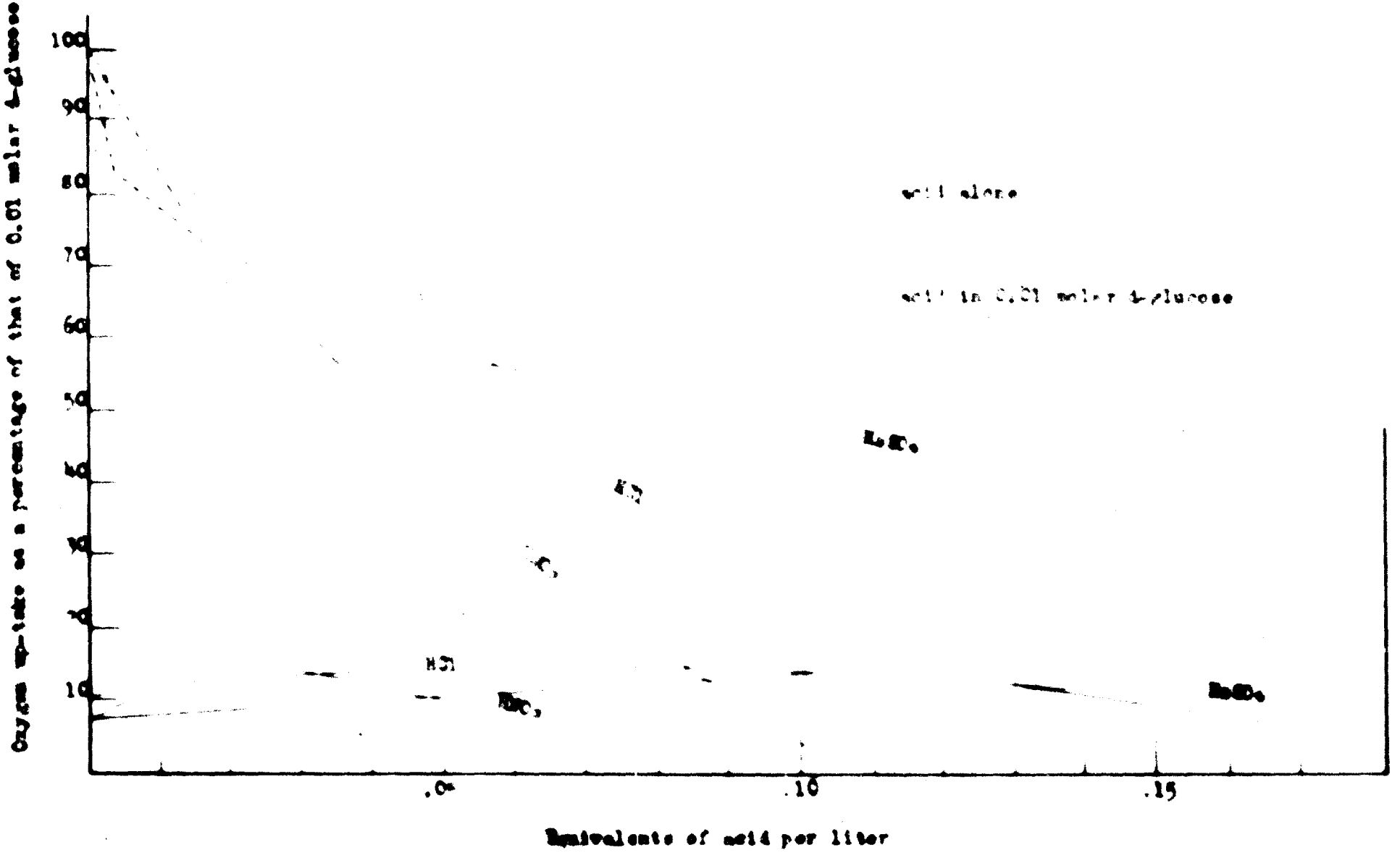

Fig. (1) The influence of inorganic acids upon the rate of oxygen up-take of Saccharomyces sanctiae

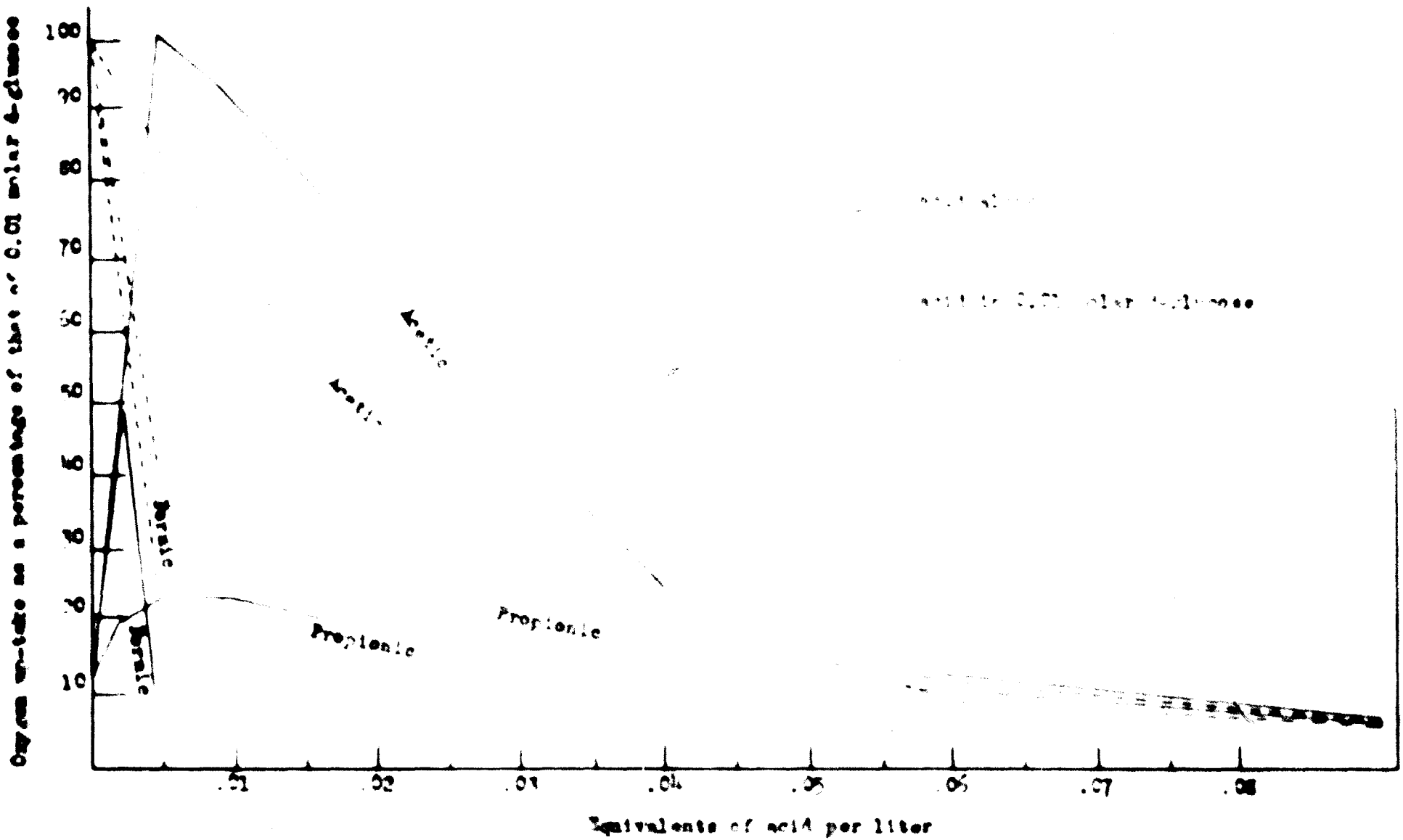

Fig. (?) The influence of saturated fatty acids upon the rate of oxygen up-take of Saccharomyces cerevisiae

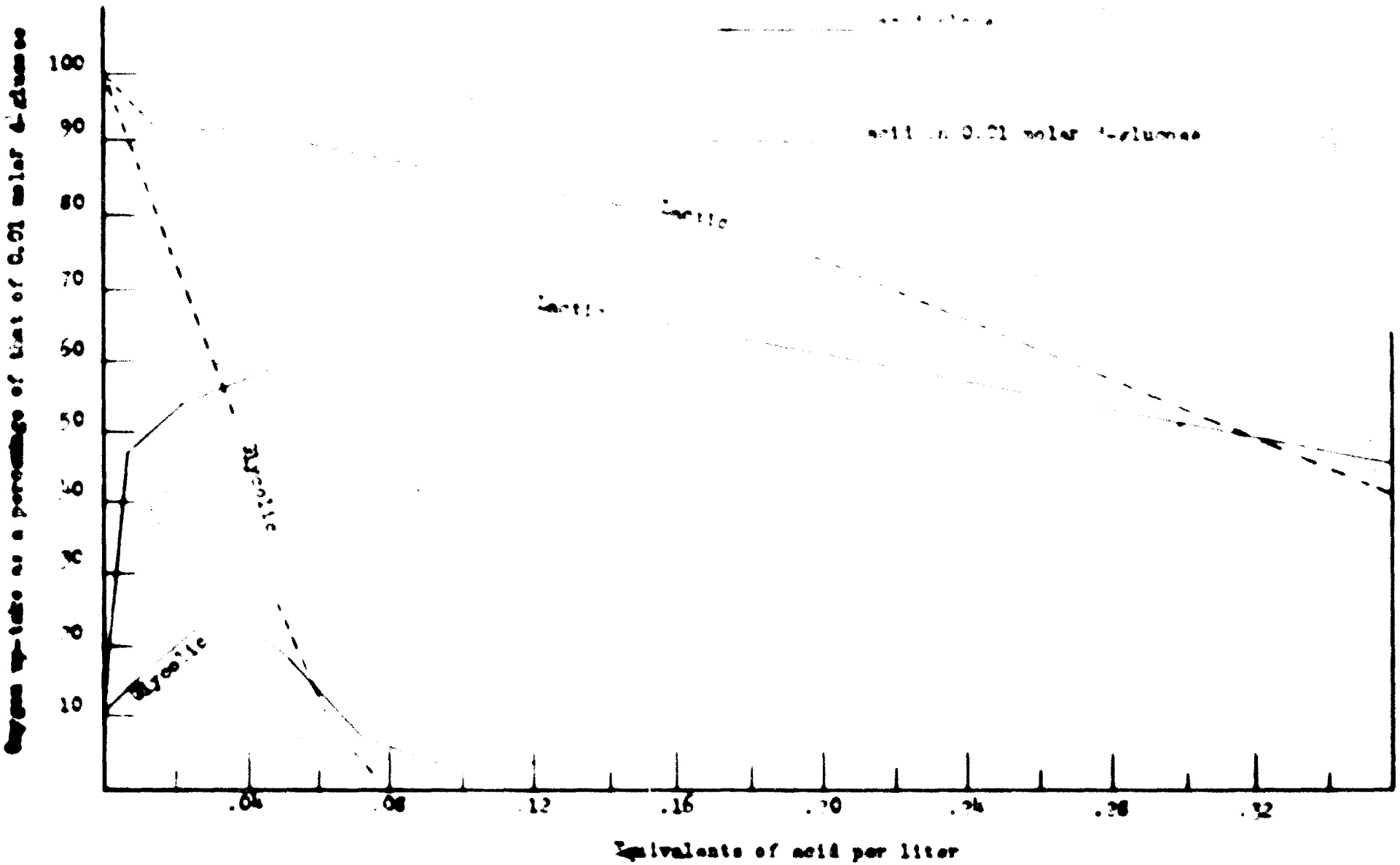

Fig. (3) The influence of hydroxy fatty acids upon the rate of oxygen up-take of Brachymonas petroleum

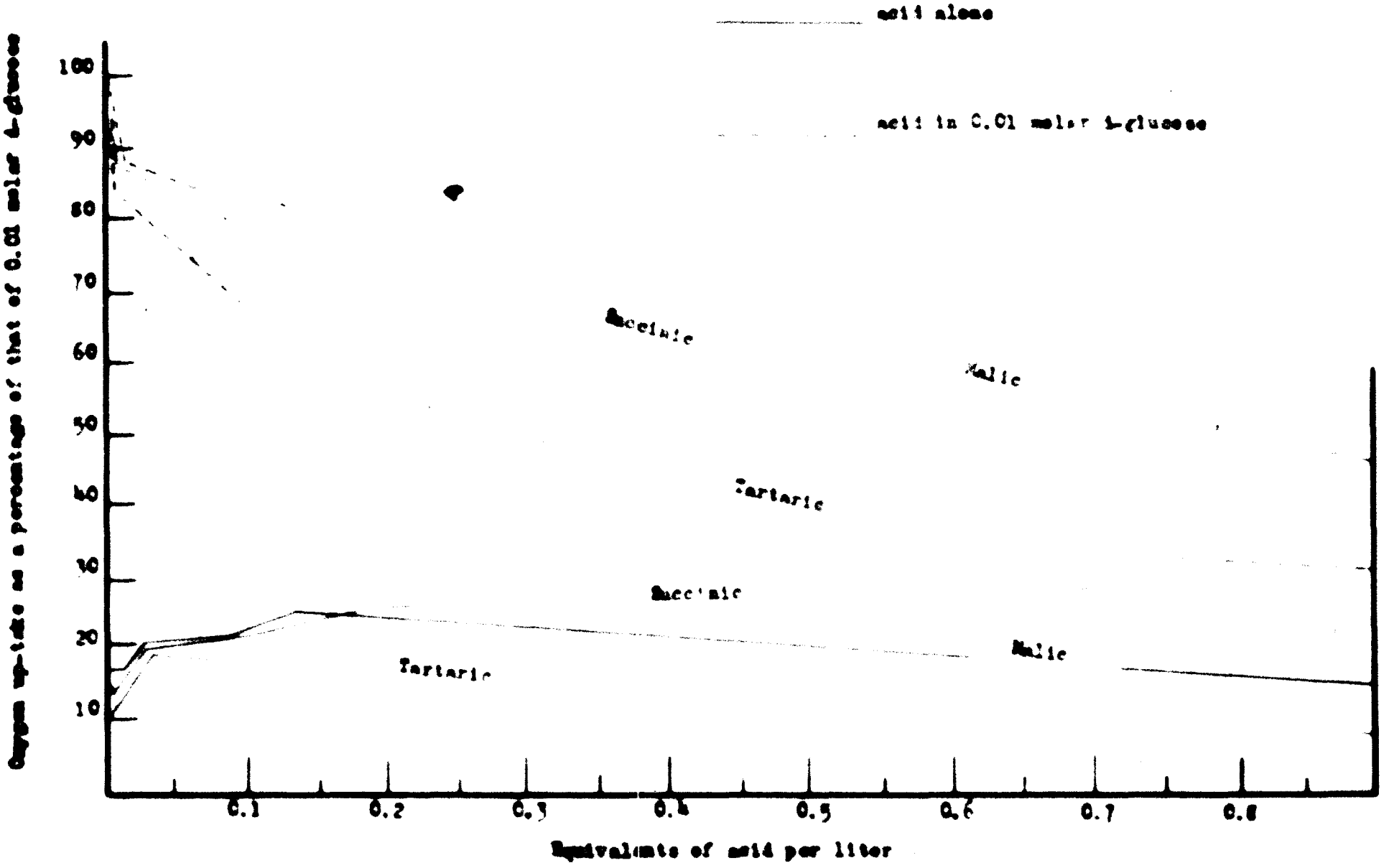

Fig. (4) The influence of four carbon dicarboxy acids upon the rate of oxygen up-take of [redacted]

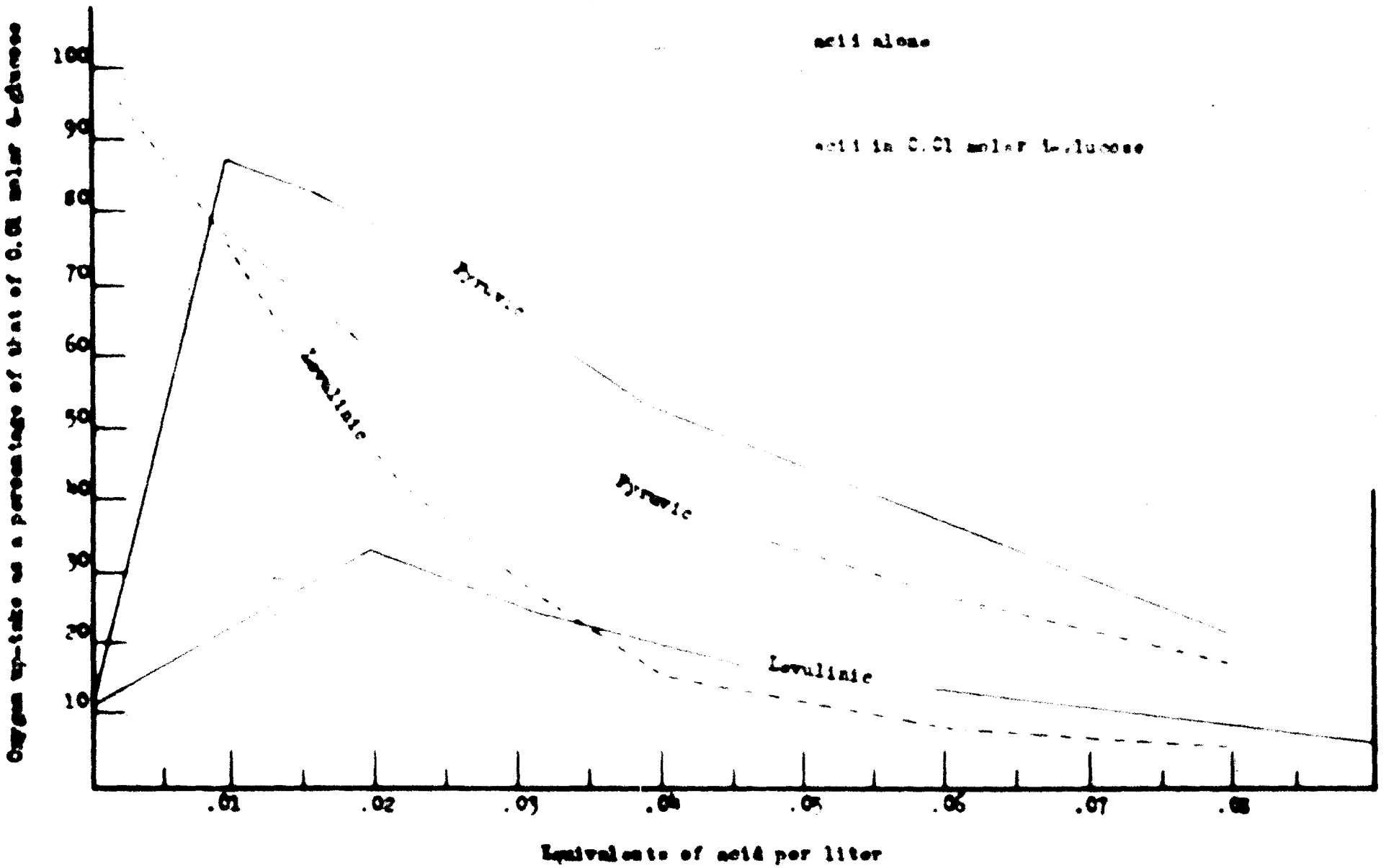

Fig. (5) The influence of keto acids upon the rate of oxygen up-take of [redacted]

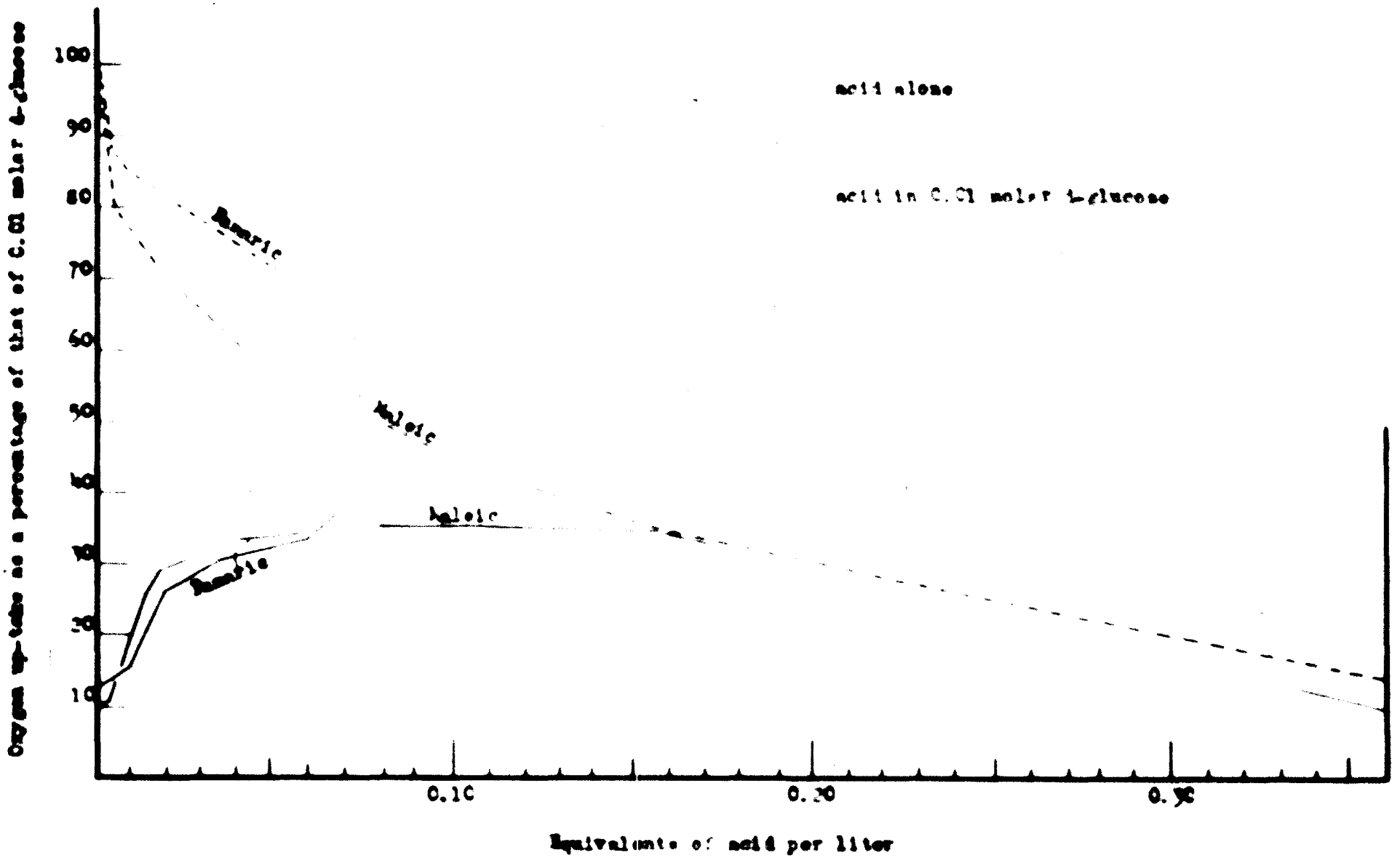

Fig. (6) The influence of ethylene dicarboxylic acids upon the rate of oxygen up-take of fumaric acid suspensions

Discussion of the results

Inorganic acids

The relative toxicity of the inorganic acids is different at the different concentrations. At 0.01 M the order of toxicity is H_2SO_4 > HCl > HNO_3 both in the presence and the absence of d-glucose. With increasing concentrations the lines cross (Fig. 1) so that at 0.10 M the order is HNO_3 > HCl > H_2SO_4, a complete reversal. The presence of d-glucose accentuates the differences between the three acids, but the comparative effects are very similar throughout.

A significant point in comparing the curves of the acids alone and in the presence of d-glucose is that with the acids alone a distinct stimulation of oxygen up-take is seen which persists in the case of hydrochloric and sulfuric acids through concentration of 0.10 normal. In the presence of d-glucose concentrations of acid as low as 0.005 M exhibit a marked inhibition of oxygen up-take. Possibly the acids exert a stimulating effect in the presence of d-glucose which is masked by the greater effect of their toxicity.

The saturated fatty acids

As seen in Fig. (2) Saccharomyces cerevisiae is able to oxidize the first three homologues of the fatty acid series. At a concentration of 0.005 M acetic acid the rate of oxygen up-take is approximately the same as from 0.01 molar d-glucose. Concentrations of acetic acid lower than 0.005 M acetic acid are apparently oxidized at the same rate as at 0.005 M. Complete data could not be obtained due to the fact that in the lower concentrations the quantity of acetic acid present is not sufficient to last for the full one hour period. Even at concentrations of 0.005 M and above an appreciable change in concentration occurs within one hour

as evidenced by the increase in pH.

In the presence of d-glucose the acetic acid probably is not oxidized. It is interesting to note that when d-glucose is present the curve for acetic acid runs appreciably lower than in the absence of d-glucose. A similar phenomenon occurs with pyruvic acid. The reason is not apparent. Propionic is much more toxic than acetic acid at low concentrations. The curves cross at 0.055 M. At higher concentrations acetic acid is slightly more toxic than propionic acid. Formic acid is by far the most toxic of the three acids.

The hydroxy fatty acids

Two acids of this series were studied, glycolic or hydroxy acetic, and lactic or hydroxy propionic acid. The hydroxy acids are completely reversed in their activity as compared to the parent compounds. Lactic acid with three carbon atoms is far less toxic than the two carbon glycolic acids. Both in the presence and absence of d-glucose and at all concentrations there is a much higher rate of oxygen up-take with lactic acid than with the same concentrations of glycolic acid.

The four carbon dicarboxy acids

In low concentrations in the absence of d-glucose and in all concentrations in the presence of the sugar the order of toxicity is tartaric > succinic > malic. There appears to be no correlation between the chemical structure of these acids and their toxicity. Tartaric acid, the most toxic, has two hydroxy groups; while malic with one hydroxy group is least toxic. Succinic acid with no hydroxy groups is intermediate.

It is impossible to tell from the data whether or not the yeast is able to oxidize these acids. In the absence of d-glucose the rate of oxygen up-take with succinic acid increases with the concentration of the

acid until it is appreciably higher than with malic acid. In the presence of d-glucose the rate of oxygen up-take is lower with succinic acid than with malic acid. This condition would be expected if the yeast were capable of oxidising succinic and not malic acid. However, this is hardly proof.

The keto acids

Pyruvic acid, as with acetic acid, shows a higher rate of oxygen up-take in the absence of sugar than in its presence. In the absence of d-glucose as was the case with acetic acid the lowest concentration capable of supporting oxidation for the full hour period showed the highest rate of oxygen up-take. As seen in Table (1?) the rate of oxidation during the first 15 minutes was the same at 0.00? as at 0.01. At a concentration of 0.00? M the rate of oxidation dropped off during the experiment due to the exhaustion of the substrate. Probably such is not the case with levulinic acid. The rate of oxidation even at the optimum concentration is only three times that in the absence of acid. The differences between the amount of oxygen up-take during a 15 minute interval are just over the limit of error and it is unsafe to draw definite conclusions.

In order to compare all of the acids studied Table (1?) and Figs. (?) and (?) have been prepared. In Fig. (?) the rate of oxygen up-take is plotted against concentration. In Fig. (?) the rate of oxygen up-take is plotted against pH.

The relative toxicity on the basis of either pH or normality is variable. If the acids are listed according to the concentration required to repress oxygen up-take to 75 per cent, the order of their arrangement is quite different from that of a list made according to the normality required to repress the oxygen up-take to 50 per cent. The order at 50 per

cent is again different from that at 25 per cent. Glycolic acid illustrates this variation. On coming a reduction to 75, 50 and 25 per cent the relative toxicity is as follows:

75 per cent	pyruvic > sulfuric > hydrochloric > malic > glycolic
50 per cent	pyruvic > glycolic > hydrochloric > malic > sulfuric
25 per cent	glycolic > pyruvic > hydrochloric > sulfuric > malic

Thus at 75 per cent glycolic is the least toxic of the five acids; at 50 per cent it is more toxic than three of the acids and at 25 per cent glycolic is the most toxic acid.

Fig. (1) The relative buffer capacities of solutions of the different salts and the ratio of oxygen up-take in 0.01 molar glutamate solutions by

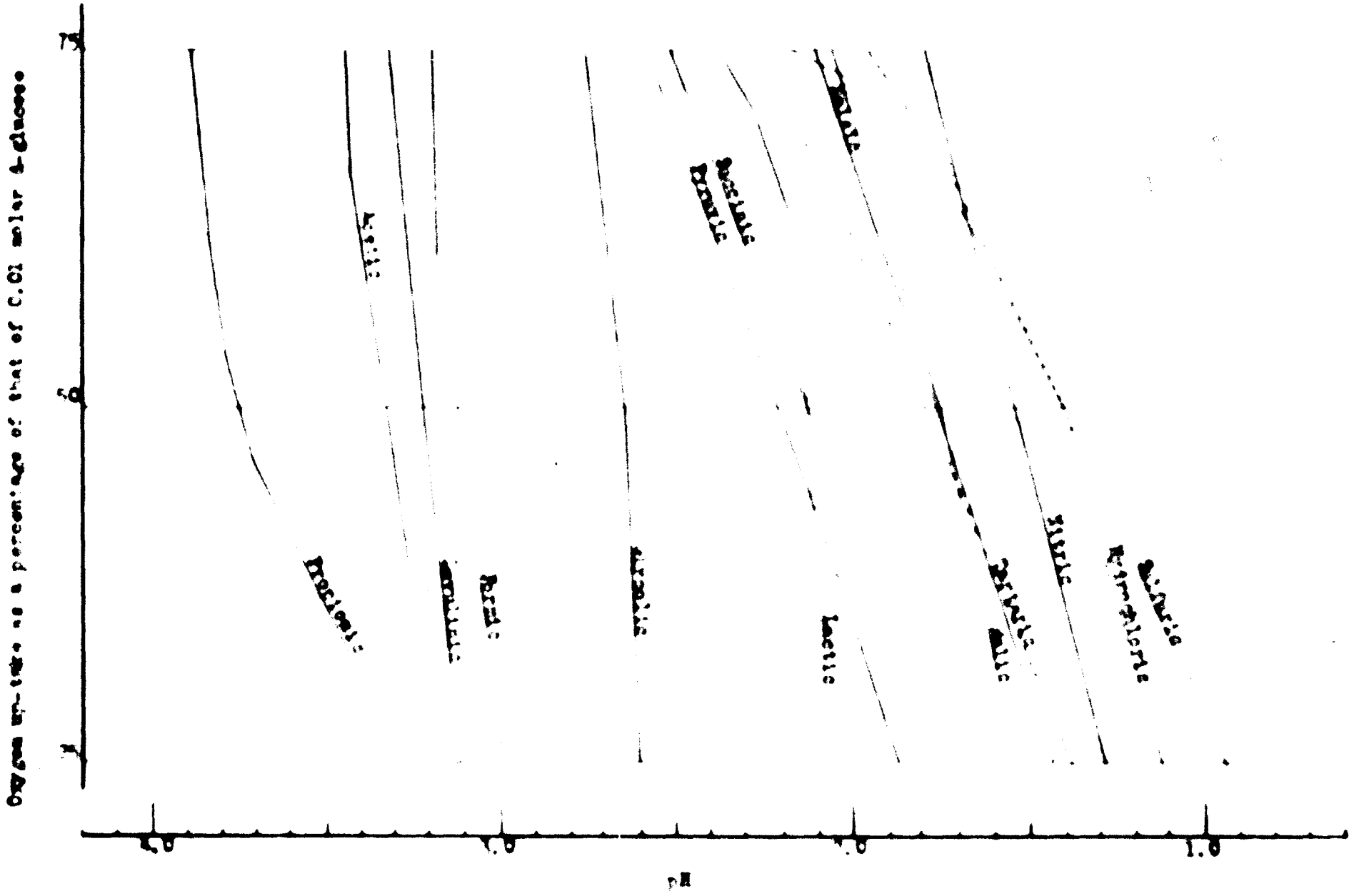

Fig. (8) The relation between the pH of the different acids and the rate of oxygen up-take in 0.01 molar d-glucose solution by Saccharomyces cerevisiae

Table (17) The amount of the various acids in 0.01 molar d-glucose solution required to reduce the oxygen uptake of Saccharomyces cerevisiae to 75, 50 and 25 per cent

	Equivalents of acid per liter			pH		
Oxygen uptake	75	50	25	75	50	25
Acid						
Nitric	.017	.03[illegible]	.0[illegible]7	1.7[illegible]	1.[illegible]	1.[illegible]
Hydrochloric	.014	.04[illegible]	.10[illegible]	1.[illegible]4	1.[illegible]0	1.1[illegible]
Sulfuric	.011	.03[illegible]	.[illegible]0[illegible]	2.19	1.[illegible]0	0.[illegible]4
Formic	.0016	.00[illegible]	.006*	3.20	3.11	3.08*
Acetic	.008[illegible]	.0[illegible]	.03[illegible]	3.4[illegible]	3.[illegible]0	3.15
Propionic	.00[illegible]	.004	.02[illegible]	3.86	3.74	3.[illegible]
Glycollic	.0[illegible]4	.0[illegible]	.05[illegible]	3.74	3.64	3.[illegible]9
Lactic	.0[illegible]	.71[illegible]	.[illegible]40	3.[illegible]8	3.1[illegible]	3.04
Succinic	.0[illegible]	.66*	—	3.[illegible]4	3.1[illegible]*	—
Malic	.0[illegible]6	.[illegible]00	1.[illegible]0	3.0[illegible]	3.[illegible]3	1.[illegible]1
Tartaric	.07	.[illegible]90	1.[illegible]*	3.[illegible]0	1.7[illegible]	1.[illegible]7
Pyruvic	.01	.0[illegible]*	.06[illegible]*	3.[illegible]6	3.[illegible]0	1.8[illegible]
Levulinic	.01	.01[illegible]*	.0[illegible]3	3.[illegible]0	3.[illegible]0	3.[illegible]0
Maleic	.01[illegible]	.06	.2[illegible]*	3.0[illegible]	1.77	1.[illegible]7

* extrapolated value

There are many other differences in relative toxicity. In fact, formic, propionic, succinic and malic are the only acids which do not change their relative toxicity between 75 and 25 per cent reduction.

To compare the relative toxicities on the basis of normality with the relative toxicities on the basis of pH the values causing a 50 per cent reduction were chosen. The acids are listed as follows (most to least toxic):

Basis of normality	Basis of pH
Formic	Propionic
Propionic	Acetic
Levulinic	Levulinic
Acetic	Formic
Pyruvic	Glycolic
Glycolic	Pyruvic
Nitric	Lactic
Hydrochloric	Succinic
Maleic	Tartaric
Sulfuric	Malic
Tartaric	Maleic
Lactic	Nitric
Malic	Hydrochloric
Succinic	Sulfuric

The toxicity of acetic acid attributable to the presence of the undissociated acid, the anion and the cation

A comparison between the toxicities of two different substances such as hydrochloric acid and acetic acid is made in a simple direct manner. The organism is exposed to various concentrations of each substance and the effect measured. The data may be plotted so that the relationship between the concentration and toxicity for each substance is clearly seen. To compare the toxicities of molecular acetic acid with its dissociation products, anion and hydrogen ion, is impossible by a direct method. The only way by which such a comparison can be made is to add various other substances to the acid solution and correlate the observed effects with the action of the added substance on the properties of the molecular acid and ions. One fault of this method is immediately apparent, any added substance can be expected to exert its own effect and the resulting change in toxicity will be due partly to the presence of the added substance and partly to the change in proportion of the molecular acid and dissociated ions. The experiments to be discussed were designed to ascertain the individual effects of the various substances which when combined with acetic acid alter the properties of molecular acid and its dissociated ions. These individual effects were then used as a basis for estimating their action when combined with acetic acid.

The properties of molecular acid, acetate ion and hydrogen ion were altered by adding the highly dissociated hydrochloric acid and sulfuric acid and by adding the highly dissociated sodium acetate.

At the concentrations involved, 0.005-0.05 N. acetic acid dissociates according to the relationship

$$C_H \times C_{Ac} = K \times C_{HAc}$$

where, C, indicates the concentration and subscripts, H, Ac, and HAc refer to the hydrogen ion, acetate ion and molecular acid respectively. The dissociation constant is designated by the letter, K, which for acetic acid at 25° C. is 1.8×10^{-5}.

If the concentration of hydrogen ions is increased through the addition of hydrochloric acid, the concentration of acetate ions decreases in accordance with the above relation. When the concentration of acetate ions is increased by the addition of sodium acetate, the hydrogen ion concentration must decrease. An idea of the approximate concentration of each constituent in the presence of hydrochloric acid or sodium acetate is seen in the following table calculated in accordance with the above relation.

	0.02 N HCl added			0.02N Na-acetate added		
Concentration of acetic acid added	Undissociated acetic acid	H ions	Acetate ions	Undissociated acetic acid	H ions	Acetate ions
0	0	.02	0	0	.00001	.02
.02	.009991	.02	.000009	.009991	.00012	.02
.03	.019982	.02	.000018	.019982	.00025	.03
.04	.039964	.02	.000036	.039964	.0005	.04
.01 (HAc alone)	.0096	.0004	.0004			

The table shows that the concentration of acetic acid is almost entirely in the molecular form in the presence of either hydrochloric acid or sodium acetate. In the presence of hydrochloric acid the concentration of hydrogen ions is the same as that of hydrochloric acid and the concentration of the acetate ions is practically zero. In the presence of so-

dium acetate the concentration of the hydrogen ions is very low.

Experiments were run to determine the individual effects of the various substances added to alter the proportion of molecular acetic acid and the acetate and hydrogen ions. In all cases the amount of yeast present was the same and the amount of d-glucose was kept constant.

The effect upon the rate of oxygen up-take was determined at concentrations of 0.01, 0.02, 0.04 and 0.08 equivalents per liter for the following substances:

Acetic acid

Hydrochloric acid

Sulfuric acid

Sodium acetate

Sodium chloride

Sodium sulfate

The results are shown in Tables IA-2C.

The three acids, acetic, hydrochloric, and sulfuric show a different degree of toxicity but they have a similar effect upon the rate of oxygen up-take in that a marked depression is noted with the addition of small amounts. Increasing amounts cause a nearly exponential increase in the rate of oxygen up-take. The sodium salts, on the other hand, are stimulating in low concentrations generally through 0.04 normal. A higher concentration was included of each salt and the results show that they are toxic at higher strengths.

The results at the higher concentrations of the salts indicate that the anions, sulfate, chloride, and acetate, vary somewhat in toxicity. At the lower concentrations, 0.01-0.08 N, there are slight differences

Table (18) The influence of combinations of the sodium salts of strong inorganic acids with acetic acid in 0.01 molar d-glucose solutions upon the oxygen up-take of Staphylococcus aureus and the pH of the solutions.

Equivalents per liter	Oxygen up-take as percentage of that of 0.01 molar d-glucose solution					pH of solutions				
Acetic acid	0	.01	.04	.06	.10	0	.01	.04	.06	.10
0	100	109.5	106.5	103.0	94.0	4.31	4.50	4.00	4.02	4.26
.005	88.0	101.0	93.[illegible]	[illegible].0	83.5	3.57	3.54	3.52	3.52	3.57
.01	77.0	85.0	80.[illegible]	73.0	79.0	3.37	3.37	3.40	3.42	3.51
.02	49.[illegible]	52.7	53.6	50.0	42.7	3.20	3.20	3.25	3.25	3.38
.04	[illegible].0	25.4	25.7	24.5	21.1	3.04	3.02	3.09	3.12	3.20
.08	4.1	5.7	9.4	7.8	7.3	2.89	3.01	2.32	2.95	3.08

Malic acid	0	.01	.04	.08	.10	0	.01	.04	.08	.10
0	100	103.5	100.0	98.0	85.7	4.31	3.92	3.97	3.80	3.58
.005	88.0	94.5	90.0	91.0	79.0	3.57	3.51	3.50	3.45	3.30
.01	73.0	79.4	76.5	77.7	66.6	3.37	3.35	3.32	3.32	3.09
.02	49.3	51.5	47.7	45.5	31.6	3.20	3.20	3.19	3.20	3.05
.04	22.0	15.4	17.4	19.8	13.7	3.04	3.00	3.00	3.00	2.97
.08	4.1	4.0	3.3	5.5	4.8	2.89	2.85	2.86	2.85	2.77

Table (13) The influence of combinations of sodium acetate with acetic acid in 0.01 molar d-glucose solutions upon the oxygen up-take of Saccharomyces cerevisiae and pH of the solutions

Equivalents per liter	Oxygen up-take as percentage of that of 0.01 molar d-glucose solution						pH of solutions					
Na-acetate / Acetic acid	0	.01	.02	.04	.08	.16	0	.01	.02	.04	.08	.16
0	100	106.0	104.0	105.0	97.0	77.0	4.31	5.57	6.07	6.10	6.17	6.46
.005	88.0	96.6	101.0	98.7	94.0	72.0	3.97	4.30	5.22	5.42	5.64	6.30
.01	73.0	92.0	82.5	90.0	81.5	68.5	3.37	4.03	4.32	5.17	5.40	6.10
.02	49.3	64.3	66.5	62.8	57.7	48.0	3.20	4.30	4.60	4.87	5.13	5.82
.04	22.0	32.5	36.2	32.2	31.8	25.2	3.04	4.00	4.30	4.64	4.80	5.43
.08	4.1	4.8	5.6	7.7	8.4	16.2	2.89	3.72	4.00	4.29	4.40	4.23

Table (?0). The influence of combinations of strong inorganic acids with acetic acid in 0.01 molar d-glucose solutions upon the oxygen up-take of Saccharomyces cerevisiae and the pH of the solutions.

Equivalents per liter	Oxygen up-take as percentage of that of 0.01 molar d-glucose solution					pH of solutions				
HNO3 / Acetic acid	0	.01	.02	.04	.08	0	.01	.02	.04	.08
0	100	60.7	75.8	67.6	55.0	3.50	2.05	1.80	1.54	1.27
.005	88.0	73.4	69.3	47.0	35.4	3.50	2.05	1.80	1.52	1.22
.01	73.0	48.5	43.7	30.1	18.4	3.39	2.07	1.80	1.53	1.22
.02	49.3	34.3	26.9	17.5	10.3	3.12	2.06	1.80	1.52	1.22
.04	22.0	13.4	11.4	7.7	5.4	2.97	2.05	1.80	1.52	1.22
.08	4.1	1.9	0	2.5	3.7	2.35	2.04	1.80	1.52	1.22

HCl / Acetic acid	0	.01	.02	.04	.08	0	.01	.02	.04	.08
0	100	60.7	73.4	72.0	25.7	3.50	1.38	1.70	1.40	1.12
.005	88.0	60.9	69.6	37.1	15.4	3.50	2.00	1.58	1.39	1.08
.01	73.0	57.2	57.3	30.0	13.5	3.39	2.02	1.65	1.38	1.07
.02	49.3	39.2	26.2	18.1	9.5	3.12	1.99	1.64	1.36	1.08
.04	22.0	10.4	13.0	10.4	5.4	2.97	1.95	1.64	1.36	1.08
.08	4.1	3.7	5.1	4.8	2.9	2.35	1.95	1.62	1.37	1.08

in the amount of stimulation caused by the different salts. Sodium sulfate and sodium acetate are almost identical in their effect; while sodium chloride is somewhat less stimulating. However, the results generally indicate that at concentrations below 0.05 M the sodium ion is the predominating factor and that the effect of the anion is insignificant in comparison.

After determining the individual effect of the three acids and their sodium salts, the influence of acetic acid in combination with the other five substances was determined. This was done with four concentrations of each substance with five concentrations of acetic acid.

Combinations of acetic acid with sodium salts

Table (18) shows the results obtained with combinations of sodium sulfate with acetic acid and sodium chloride with acetic acid. In the case of sodium sulfate, which is stimulating when present alone in concentrations through 0.05 M, it is seen that a similar condition exists with combinations of 0.01, 0.04, and 0.05 M sodium sulfate and 0.005, 0.01, 0.02, 0.04, 0.05 M acetic acid. That is, the stimulating action of sodium sulfate counteracts the toxic effect of acetic acid. The same results are seen with combinations of sodium chloride and acetic acid. The presence of an amount of sodium chloride which is stimulating alone, when combined with acetic acid results in a lowered toxicity as compared to the same concentration of acetic acid alone.

Similarly in combination with acetic acid, an amount of sodium chloride or sodium sulfate, which is toxic alone, exhibits an increased toxicity compared to that of the same concentration of acetic acid alone.

Combinations of acetic acid with stimulating concentrations of sodium acetate have an effect upon oxygen up-take which is qualitatively the same as with sodium sulfate or sodium chloride. Combinations of acetic acid with stimulating concentrations of sodium acetate are less toxic than acetic acid alone. Acetic acid - sodium acetate combinations in which the concentration of sodium acetate is high enough so that by itself it would be slightly toxic, 0.05 M, show a combined effect that is less toxic than acetic acid alone. When the concentration of sodium acetate is greatly increased, 0.10 M, in the combinations, the combined effect is only slightly more toxic than acetic acid alone.

Combinations of acetic acid with strong inorganic acids

The combination of acetic acid with strong inorganic acids, both of which are toxic in themselves, shows a greater toxicity than does either component alone. Hydrochloric acid is more toxic than sulfuric acid and the combinations of acetic acid with hydrochloric acid are more toxic than combinations of acetic acid and sulfuric acid.

The results of the experiments so far discussed may be summarized as follows:

(1) In low concentrations the sodium salts of acetic, sulfuric and hydrochloric acids have a slight stimulating effect. The fact that the degree of stimulation varies only slightly with the anion indicates that the action is primarily due to the sodium ion.

(2) Concentrations of sodium sulfate and sodium chloride which are stimulating when used alone are stimulating when present in combination with acetic acid. Concentrations of the same salts which are toxic when used alone are toxic when present in combination with acetic acid.

(3) Sodium acetate when used in combination with acetic acid shows a greater stimulating action than would be expected from the presence of the sodium ion.

(4) Hydrochloric and sulfuric acids in combination with acetic acid are more toxic than acetic acid alone.

The effect of the hydrogen ion

In tables (15-2C) is shown the pH of each combination.

The addition of sodium sulfate or sodium chloride in concentrations of 0.01, 0.04, or 0.06 normal to acetic acid solutions has no effect upon the concentration of hydrogen ion. The presence of these salts does not alter the proportions of molecular acetic acid, acetate ion and hydrogen ion and the effect of the salts may be considered as that due solely to their action on the yeast. The presence of sodium acetate in combination with acetic acid markedly alters the pH from that of acetic acid alone. Thus the effect of sodium acetate may be considered as due to the action of sodium acetate upon the yeast and also to the change in the proportions of molecular acid, acetate ion and hydrogen ion.

The difference between the effect of sodium chloride and sodium sulfate and the effect of sodium acetate is more clearly seen in Table ( ). Here the oxygen up-take of the various combinations has been "corrected" for the individual effects of the sodium salts upon the yeast. It is assumed that the effect of the action of the salts directly upon the yeast is the same in the presence of acetic acid as in its absence. There is no real basis for making such an assumption but the results appear to justify it. The values were "corrected" in the following manner: the oxygen up-take of each concentration of salt was arbitrarily assigned the value of 100 and the values listed under it calculated to

Table (21) The influence of combinations of the sodium salts of sulfuric, hydrochloric and acetic acids in 0.01 molar d-glucose solutions. "corrected" for the presence of the salts, upon the oxygen uptake of [illegible]

"Corrected" oxygen up-take

Equivalents per liter acetic acid						Average 0.01, 0.02, 0.04 0.08 M salt
Na_2SO_4	0	.01	.02	.04	.08	.16
0	100	100	100	100	100	100
0.005	88	92	93	89	89	91
0.01	73	73	75	71	84	73
0.02	49	46	50	49	45	49
0.04	22	23	24	25	22	24
NaCl	0	.01	.02	.04	.08	.16
0	100	100	100	100	100	100
0.005	85	91	90	93	92	91
0.01	73	77	77	79	78	78
0.02	49	50	46	47	37	46
0.04	22	15	17	21	16	18
[illegible]	0	.01	.02	.04	.08	.16
0	100	100	100	100	100	100
0.005	88	83	97	94	97	94
0.01	73	85	73	85	84	83
0.02	49	59	64	60	60	61
0.04	22	31	34	31	33	33

the same basis. For instance, the oxygen up-take for 0.01 normal sodium sulfate was 109.5. All values listed under that concentration were multiplied by the factor 100/109.5. Thus the value for 0.01 normal sodium sulfate in the absence of acetic acid becomes 100 and the succeeding values become percentages of the oxygen up-take compared to that of 0.01 normal sodium sulfate.

In the same manner the values for the rate of oxygen up-take of combinations of sulfuric acid with acetic acid and hydrochloric acid with acetic acid have been "corrected" for the action of the inorganic acid upon the yeast. The "corrected" data are listed in Table (22).

Except in a few cases the values at any one concentration of acetic acid are the same regardless of whether the concentration of sulfuric acid in the combination is 0.01, 0.02, or 0.04. This is also true with combinations of 0.01, 0.02, .04 HCl. The fact that the "corrected" values are practically constant for different concentrations of the same substance makes it possible to average the values so that they may be more readily compared, as follows:

Equivalents per liter Acetic acid	Average "corrected" oxygen up-take in combinations of acetic acid with various substances					
	Control	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0	100	100	100	100	100	100
0.005	88	91	91	94	75	69
0.01	73	73	78	83	59	53
0.02	49	49	46	61	37	36
0.04	22	24	18	33	15	17

The average "corrected" values for sodium sulfate and sodium chloride are very nearly the same as for acetic acid alone. This indicates that these two salts exert the same effect in the presence of acetic acid as in its absence. This would be expected if their effect were upon the yeast only.

Table (22) The influence of combinations of sulfuric and hydrochloric acetic acid in 0.01 molar d-glucose solutions, "corrected" for the presence of the inorganic acids, upon the oxygen up-take of Saccharomyces cerevisiae

Corrected oxygen up-take

Equivalents per liter acetic acid	H_2SO_4 0	.01	.02	.04	.08	Average of 0.01, 0.02, 0.04 inorganic acid
0	100	100	100	100	100	100
0.005	88	76	78	70	65	75
0.01	73	59	58	60	52	59
0.02	49	41	36	35	33	37
0.04	22	13	16	15	10	15

	HCl 0	.01	.02	.04	.08	
0	100	100	100	100	100	100
0.005	83	75	69	64	60	69
0.01	73	59	52	48	44	53
0.02	49	36	36	35	37	36
0.04	22	13	17	20	21	17

The "corrected" values for combinations containing sodium acetate, sulfuric acid, or hydrochloric acid are considerably different from those of acetic acid alone. This suggests that some factor is involved other than the effect of these substances upon the yeast.

It appears unlikely that this factor is concerned with the concentration of undissociated acetic acid. It was pointed out above that either sodium acetate or strong inorganic acids depress the ionization of acetic acid almost completely. The acetic acid is dissociated to the extent of only six per cent in 0.005 N solution. It seems most improbable that a slight change of six per cent in the amount of undissociated acid would cause an appreciable change in toxicity. The remaining correlation is that of the concentration of hydrogen ion. As seen by the behavior of sulfuric and hydrochloric acids, the hydrogen ion is toxic. The addition of sodium acetate to acetic acid solutions decreases the concentration of hydrogen ion and decreases the toxicity. The addition of sulfuric or hydrochloric acid to acetic acid solutions increases the concentration of hydrogen and the toxicity. It has been shown that the ions present other than the hydrogen ion have the same proportional effect on oxygen up-take whether acetic acid is present or not. It has been shown that under the conditions of the experiments the change in the concentration of the undissociated acetic acid is slight and that the slight change is not a logical explanation of the experimental results.

There remains the fact that with a constant concentration of molecular acetic acid the hydrogen ion exerts a toxic action greater than that attributable to the hydrogen ion in the absence of acetic acid.

The manner in which the two factors, molecular acetic acid and hydrogen ion, operate so as to exert a total action greater than the sum of their individual effects is a matter of conjecture. Many possibili-

ties suggest themselves. One of the more plausible of these is that one factor increases the permeability of the yeast cells thereby permitting the second substance to act more effectively. A significant point is that the synergistic effect is constant over a considerable range in the concentration of sulfuric or hydrochloric acid. Quadrupling the concentration of the inorganic acid changes only slightly the amount of toxic action not attributable to the components. The same thing is true with combinations of acetic acid with sodium acetate; the difference between the combined effects and the sum of the individual effects is very nearly the same when 0.01 equivalents of sodium acetate are added as when 0.04 equivalents are added.

Summary

The influence of 15 acids upon the rate of respiration of [illegible] was determined with the Warburg apparatus. Various concentrations of each acid were used covering a range from the lowest strength sufficient to produce a measurable effect to a strength which almost completely inhibited oxygen up-take.

The order of toxicity was found to vary with the degree of action caused. When compared on the basis of the amount of acid which caused a 25 per cent decrease in oxygen up-take, the order of their increasing equivalent concentrations or decreasing toxicity was as follows: formic, propionic, pyruvic, levulinic, acetic, sulfuric, hydrochloric, maleic, glycolic, nitric, fumaric, tartaric, lactic, succinic, and malic. When compared at the strength causing a 75 per cent decrease in oxygen up-take the order of decreasing toxicity was, formic, propionic, levulinic, acetic, glycolic, pyruvic, nitric, hydrochloric, sulfuric, maleic, lactic, malic, and tartaric.

When the pH values causing a certain inhibition of oxygen up-take were used as a basis of comparison, the order of toxicity varied with the degree of inhibition and the sequence was quite different from that based on the equivalent concentrations.

An attempt to determine the toxicity of acetic acid and its dissociated ions was made by combining strong inorganic acids and sodium acetate with acetic acid. The combined action of acetic acid and strong inorganic acids was more and the combined action of acetic acid and sodium acetate was less toxic than the sum of their individual effects. Furthermore, the differences between the combined effects and the sum

of the individual effects changed only very slightly when the concentration of the substance added to the acetic acid was increased as much as four times.

It is suggested that the action of acetic acid is due primarily to the undissociated acid and that this primary action is enhanced by the hydrogen ion.

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