ACTION OF ACETIC ACID ON FOOD SPOILAGE MICROORGANISMS¹

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This investigation was initiated with the purpose of studying the effect of acetic acid on certain microorganisms related to food spoilage with the hope of securing results of practical value. The main reasons for the use of acetic acid as a food preservative are its toxicity value, commercial availability, and low cost.

The toxic effect on microorganisms of an increased hydrogenion concentration in the substrate is well established. Acetic acid and certain other organic acids appear to have a toxicity in excess of that which could possibly be due to the pH alone. The consensus of authorities seems to favor the theory that it is the undissociated molecule which is toxic. Kahlenberg and True (1896) found that the undissociated acetic acid molecule was toxic. Winslow and Lockridge (1906) found that acetic and benzoic acids were fatal to typhoid and colon bacilli at a strength at which these acids were but slightly dissociated. Wolf and Shunk (1921) concluded that the hydrogen-ions alone were not responsible for the toxicity of acetic acid. Bach (1932) states that at an interval where the pH has no importance, the undissociated part of the lactic acid is the active factor, although generally the hydrogen-ions control the antiseptic effect. In another paper (1932a) the same investigator suggested that the antiseptic action of formic, acetic, propionic, and butyric acids was connected with their influence on surface tension. Foster (1920) also contributed to the idea that the toxic effect was due to the

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whole molecule rather than to hydrogen-ions alone. Kirby. Frey, and Atkin (1935, 1936) found that acetic acid, either chemically pure or as vinegar, had a marked influence on the growth of bread molds, particularly Aspergillus niger. These investigators observed that at the same pH, greater inhibition was obtained with an increasing concentration of acetic acid. Collett (1919, 1921) stated that, although the hydrogen-ions are important in solutions of equal pH, butyric and acetic acids were more toxic than hydrochloric and other strong acids. Tekelenberg (1927) demonstrated that the alkali salts alone of several organic acids had no marked bactericidal effect. Cohen and Clark (1919) concluded that the limiting pH is a zone rather than a value and the chief effect of reaction in growth rate studies seems to be confined to the extremes. They suggest that the toxic action of acetic acid may be due to the free acetate radical exerting a synergic effect on the disinfecting power of the hydrogen ion.

Kolthoff (1925) stated that the growth of bacteria in cultures is halted not by the hydrogen-ions, but by the formation of undissociated acids. Bach (1932) has stated that generally the hydrogen-ions control the antiseptic effect but that the undissociated part of lactic acid is the active factor when the pH value is such as to be unimportant.

EXPERIMENTAL

The studies in this paper include: (1) A determination of the concentrations of acetic acid in broth media which will destroy and inhibit the growth of the test organisms during the incubation period; (2) An investigation of the effect of acetic acid on the thermal death points of the test organisms; and (3) A comparison of acetic, lactic, and hydrochloric acids as to their inhibitory and toxic properties on a typical yeast, mold, and bacterium. Hydrogen-ion activities as well as total titratable acidity are considered in these experiments.

INDEX ORGANISMS

The test microorganisms used in this investigation were: Salmonella aertrycke, Staphylococcus aureus, Phytomonas phaseoli,

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Bacillus cereus, Bacillus mesentericus; Saccharomyces cereviseae Lister; and Aspergillus niger.

METHODS

Inhibiting and lethal concentrations of acetic acid were determined by a somewhat modified phenol-coefficient technic whereby known quantities of acid and medium were mixed in a series of test tubes, inoculated and incubated at suitable temperatures. The comparative effects of acetic, lactic, and hydrochloric acids on the total destruction and reduction of numbers of bacteria and yeast were determined by a 15-minute contact of the test organism in an acid solution followed by a standard quantitative plating on nutrient agar for the bacteria and on glucose agar for the veast. The mold was cultivated in flasks of glucose broth and after the incubation period the mat was carefully washed, dried and weighed. Thermal death points were studied in test tubes in media whose hydrogen-ion concentrations were adjusted by acetic acid. Suspensions of spore-forming bacteria contained spores as well as vegetative cells. Inoculations of all suspensions were made by means of a standard four-millimeter loop.

RESULTS

Table 1 summarizes the data obtained in determining concentrations of acetic acid which inhibited growth and those that were lethal to the organisms in tubes of broth. The growth of *Salmonella aertrycke* in nutrient broth, containing acetic acid, was inhibited at pH 4.9 but the organism was still viable. At pH 4.5 this organism was destroyed. *Aspergillus niger* was slightly less resistant to the action of acetic acid than was the yeast. Both yeast and mold were markedly more resistant to the acid than were any of the bacteria studied. Where the inhibiting and lethal acidities were the same, slight differences might have been shown had the pH increments been smaller.

Thermal death point studies

Thermal death point studies on microorganisms are of considerable value, especially to the canner in determining processing times and temperatures for the preservation of various food products. The addition of a small amount of acetic acid may aid in reducing the time or temperature required to sterilize the product commercially. Table 2 is a summary of results obtained when the pH of the broth was adjusted with acetic acid. The effect of an acid reaction in reducing the thermal death temperature was most marked in the case of the aerobic spore-formers, *Bacillus cereus* and *B. mesentericus*. The addition of small amounts of acetic acid to the medium did not affect the thermal death points of the yeast or mold.

TABLE 1

Inhibiting and lethal acetic acid concentrations for microorganisms

ORGANISM	INHIBITING pH ⁺	INHIBITING ACIDITY	LETHAL pH†	LETHAL ACIDITY
		per cent		per cent
Salmonella aertrycke	4.9	0.04	4.5	0.09
Staphylococcus aureus	5.0	0.03	4.9	0.04
Phytomonas phaseoli	5.2	0.02	5.2	0.02
Bacillus cereus	4.9	0.04	4.9	0.04
Bacillus mesentericus	4.9	0.04	4.9	0.04
Saccharomyces cereviseae	3.9	0.59	3.9	0.59
Aspergillus niger	4.1	0.27	3.9	0.59

* The pH at which no visible growth occurred yet the microorganism remained viable.

† The pH at which total destruction took place.

Comparison of acetic, lactic, and hydrochloric acids

To determine whether the degree of toxicity of added acid was due to the hydrogen-ion activity, to the organic or inorganic nature of the acid, or to some factor peculiar to acetic acid, experiments were conducted to compare the effects of acetic, lactic, and hydrochloric acids. Salmonella aertrycke, Saccharomyces cereviseae Lister, and Aspergillus niger were used. The results with Salmonella aertrycke are shown in table 3. This organism was inhibited in broth at pH 4.9 and killed in broth at pH 4.5 containing 0.04 and 0.08 per cent acetic acid, respectively. Salmonella aertrycke grew in broth with a pH 4.5, having a lactic acid content of 0.06 per cent and was inhibited and killed when the lactic acid was increased to 0.12 per cent with a pH of 4.0. An acidity of 0.03 per cent of hydrochloric acid with a pH value of 4.0 inhibited growth. Toxicity and inhibition in the case of hydrochloric acid seem to be due to the hydrogen-ion concentration and not to the whole molecule as in the case of acetic acid. Lactic acid is the least effective in inhibiting growth of *S. aertrycke* under the conditions of this experiment.

TABLE	2
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Effect of pH of the medium on the thermal death point of microorganisms, exposure period, 10 minutes

ORGANISM	pH VALUE	LETHAL	TEMPERATURE
		°C.	°F.
<u>.</u>	6.6	55	131
Saimonella aeritycke	5.0	50	122
а	6.6	65	149
Staphylococcus aureus	5.5	60	140
	6.6	55	131
Phytomonas phaseoli	5.7	50	122
D	6.6	100	212
Bacillus cereus	5.5	60	140
D	6.6	100	212
Bacillus mesentericus	5.5	60	140
~ ~ (6.8	60	140
Saccharomyces cereviseae	4.5	60	140
ſ	6.8	60	140
Aspergillus niger	5.0	60	140
	4.5	60	140

Reaction	adjusted	by	acetic	acid
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Table 4 shows the results obtained with yeast. Cells of Saccharomyces cereviseae were destroyed in glucose broth tubes of pH 3.9 or lower with an acetic acid content of 0.59 per cent or more. The toxic limit of lactic acid for this yeast was considerably higher, being between 1.54 and 3.08 per cent. Again, less hydrochloric acid destroyed the test organism but the hydrogen-ion activity in the limiting tube with hydrochloric acid was 2.2 as compared with the limiting pH of 3.9 when acetic acid was used.

ACIDITY OF BROTH COMPLETE TOTAL DESTRUCTION OF BACTERIA. INHIBITION MEDIUM OF GROWTH. Calculated total acidity pН 48 HOURS 48 HOURS per cent 4.0 0.33 4.3 0.17 - - + + + + 4.5 0.08 4.9 0.04 Broth + acetic acid... -+ + 5.20.025.6 0.01 5.7 0.005 +- - - + + + + 3.2 0.46 - - + + + + 3.6 0.234.0 0.12 Broth + lactic acid... 0.06 4.55.0 0.03 5.5 0.01 6.0 0.007 0.27 1.5 --++ 2.10.13 3.1 0.07 Broth + HCl acid.. 0.03 4.0 4.7 0.02 +5.2+0.008 +5.8 0.004 +

 TABLE 3

 Comparative inhibitory effect of acetic, lactic, and hydrochloric acids in nutrient broth on the growth of Salmonella aertrycke

The inoculation consisted of one loopful of a 48-hour broth culture of Salmonella aertrycke.

Table 5 shows that in acidity ranges similar to those of the yeast experiment, the spores of *Aspergillus niger* were killed when the hydrogen-ion concentration of the medium adjusted with acetic acid was pH 3.9 and the total acidity was 0.59 per cent. At pH values of 4.0 and 4.1 corresponding to 0.37 and 0.27 per cent acidity, respectively, the mold spores did not vegetate but

did maintain their viability. When the medium was adjusted with 0.27 per cent hydrochloric acid resulting in a pH of 1.6, the spores not only survived but also developed into vegetative mycelia. With this acid a pH value of 1.2 and an acidity of 0.38 per cent was required to prevent growth. Aspergillus niger tolerated

	ACIDITY	OF BROTH	COMPLETE INHIBITION	TOTAL
MEDIUM -	pH	pH Calculated total acidity		OF YEAST CELLS 48 HOURS
		per cent		
()	3.6	1.18	-	-
	3.7	0.74		-
Proth L costic soid	3.9	0.57	_	-
	4.0	0.37	+	+
	4.4	0.18	+	+
	4.7	0.09	+	+
(2.0	12.32	_	_
	2.2	6.16	-	-
Broth lostic soid	2.5	3.08	-	-
Broth + lactic acid	2.8	1.54	+	+
	3.2	0.77	+	+
	3.4	0.39	+	+
(1.6	0.27	_	_
	2.2	0.13	_	_
Broth + HCl acid	3.3	0.07	+	+
	4.1	0.03	+	+
	4.8	0.02	+	+

TABLE 4

Comparative inhibitory effect of acetic, lactic, and hydrochloric acids in glucose broth on the growth of Saccharomyces cereviseae Lister

The inoculation consisted of one loopful of a 48-hour broth culture of Saccharomyces cereviseae Lister.

a relatively high lactic acid content in the medium, growing abundantly at 3.08 per cent. Here, the limiting pH of 2.2 was higher than that of the hydrochloric acid but lower than that of acetic acid. Because acetic acid was toxic at a lower hydrogenion activity and especially in the case of mold at a lower total acidity than either lactic or hydrochloric acid, the toxicity of acetic acid is not entirely a function of pH but due more to the nature of the acid itself.

Comparative quantitative studies

The comparative effects of different concentrations of acetic, lactic, and hydrochloric acids on the extent of growth of Sal-

	ACIDITY	OF BROTH	COMPLETE	TOTAL
MEDIUM	pH	Calculated total acidity	OF GROWTH, 48 HOURS	OF MOLD, 48 HOURS
		per cent		
	3.6	1.18	-	
	3.7	0.74	-	-
	3.9	0.59	-	-
Broth + acetic acid	4.0	0.37	-	+
	4.1	0.27	-	+
	4.4	0.18	+	+
l	4.7	0.09	+	+
(2.0	12.32	_	
	2.2	6.16	-	-
Durath 1 location and	2.5	3.08	+	+
Broth + lactic acid	2.8	1.54	+	+
	3.2	0.77	+	+
l	3.4	0.39	+	+
(1.2	0.38	_	-
	1.6	0.27	+	+
Broth + HCl acid	2.2	0.13	+	+
	3.3	0.07	+	+
	4.1	0.03	+	+

TABLE 5

Comparative inhibitory effect of acetic, lactic, and hydrochloric acids in glucose broth on the growth of Aspergillus niger

The inoculation consisted of a loopful of a water suspension containing about 200 spores.

monella aertrycke, Saccharomyces cereviseae, and Aspergillus niger were studied. Table 6 shows that acetic acid was the most toxic acid to S. aertrycke on the basis of hydrogen-ion activity. It was slightly better than lactic acid at an equivalent pH value and was much more toxic than hydrochloric acid when in contact with the test organism for 15 minutes. It is true that less hydro-

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chloric acid was required to increase the hydrogen-ion activity to its toxic limit than in the case of either lactic or acetic acids. However, both the latter acids were potent at a higher pH than the hydrochloric acid. The percentage reduction in numbers of

TABLE	6
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Comparative effect of acetic, lactic, and hydrochloric acids on the survival of Salmonella aertrycke in water

	ACIDITY OF SOLUTION			REDUCTION IN	
TTPE OF SOLUTION	pH	Calculated total acidity	TOTAL COUNT	NUMBERS	
		per cent	bacteria per ml.	per cent	
(2.9	0.33	0	100.00	
	3.1	0.17	420	98.83	
	3.3	0.08	1,800	95.00	
water + acetic acia	3.5	0.04	3,600	90.00	
	3.7	0.02	7,200	80.00	
	3.9	0.01	13,900	61.39	
(2.5	0.46	0	100.00	
	2.7	0.23	0	100.00	
	2.9	0.11	14	99.96	
Water + lactic acid	3.0	0.06	200	99.44	
	3.2	0.03	1,500	95.83	
	3.4	0.01	6,000	83.33	
l	3.6	0.007	16,200	55.00	
(1.3	0.27	0	100.00	
	1.6	0.13	0	100.00	
	1.9	0.07	0	100.00	
Water + HCl acid	2.1	0.03	750	97.92	
	2.4	0.02	12,000	66.67	
l	2.7	0.01	20,400	43.33	
Water only	6.2	0.0	36,000		

Contact period, 15 minutes

The inoculation per 5 ml. of solution contained 120,000 bacteria.

surviving bacteria was calculated on the basis of the number of S. aertrycke present in the distilled water.

The effect of the three acids on the yeast, S. cereviseae, after a 15-minute contact period is shown in table 7. The difference • in limiting acidities was much more marked with this organism than with S. aertrycke. For complete destruction of the yeast cells 2.94 per cent acetic acid at a pH of 2.6 was required as compared with 5.25 to 10.49 per cent lactic acid at a pH value of about 1.7 and 0.92 per cent hydrochloric acid at a pH value of

	ACIDITY O	F SOLUTION		PEDICTION IN
TYPE OF SOLUTION	pH Calculated total acidity		TOTAL COUNT	NUMBERS
		per cent	cells per ml.	per cent
()	2.6	2.94	0	100.00
	2.8	1.47	90	91.00
Watan I antia and	2.9	0.74	311	68.90
water + acetic acid	3.1	0.37	450	55.00
(3.2	0.18	560	44.00
	3.4	0.09	690	31.00
(1.7	10.49	0	100.00
	1.8	5.25	9	99.10
W. 4	2.0	2.62	134	86.60
water + lactic acid	2.1	1.31	340	66.00
	2.3	0.66	380	62.00
l	2.6	0.33	370	63.00
	0.2	3.68	0	100.00
11	0.6	1.84	0	100.00
	0.7	0.92	0	100.00
water + HCI acid	1.1	0.46	146	85.40
	1.3	0.23	470	53.00
l	1.6	0.12	470	53.00
Water only	6.2	0.0	1,000	

Saccharomyces	cereviseae	Lister in	water
Contact	period. 15	<u><u><u>ó</u></u> minutes</u>	

TABLE 7
Comparative effect of acetic, lactic, and hydrochloric acids on the survival of

The inoculation per 5 ml. of solution contained 4500 cells.

0.7. There was some survival of yeast cells when the hydrogenion activity of the lactic acid solution was pH 1.8 and of the hydrochloric acid solution was 1.1. Here, although less lactic acid than acetic acid was required to give the same pH value, a smaller content of acetic than lactic acid was required for the same degree of toxicity. A quantity of the strongly dissociated hydrochloric acid smaller than in the case of either of the other two weakly dissociated acids was required to reduce the number

	TABLE 8	
Comparative effect	of acetic, lactic, and hydrochloric acids on the growth of Aspergillus niger of glucose broth	λf

	INITIAL	ACIDITY	FINAL ACIDITY		NBY	
MEDIUM	pH	Titratable acidity	рН	Titratable acidity	WEIGHT	
		per cent		per cent	mgm.	
[4.1	0.27	4.1	0.27	0.0	
	4.3	0.21	4.3	0.21	17.3	
	4.4	0.16	7.1	0.03*	162.0	
Broth + acetic acid	4.5	0.15	7.6	0.03	147.8	
l	4.7	0.10	7.6	0.03	133.3	
	5.2	0.07	7.6	0.03	120.5	
ſ	2.2	4.84	2.2	5.43	0.0	
	2.3	4.16	2.3	4.56	16.7	
	2.4	3.48	2.8	1.24	495.7	
	2.5	2.46	3.2	0.24	486.3	
Broth + lactic acid	2.6	1.91	4.2	0.06	424.0	
	2.7	1.80	7.1	0.04	375.4	
	2.8	1.21	7.4	0.04	311.0	
l	3.1	0.72	7.6	0.04	206.0	
ſ	1.3	0.35	1.3	0.36	0.0	
	1.4	0.30	1.4	0.31	8.0	
	1.6	0.25	1.6	0.26	17.5	
Broth + HCl acid	1.8	0.21	1.8	0.21	41.7	
	2.3	0.12	7.5	0.04	163.8	
	2.9	0.10	7.6	0.04	136.7	
Broth control	6.8	0.03	7.6	0.04	128.1	

The inoculation consisted of 0.1 ml. of a water suspension containing about 2000 spores.

* At final pH values of 7.0 or higher the small apparent acidity represents a blank which is not subtracted in this table.

of surviving cells but the toxic action of hydrochloric acid manifested itself at a relatively much lower pH value than that of the other two acids, especially acetic acid.

Plate 1 shows the comparative effects of acetic, lactic, and hydrochloric acids on the growth of Aspergillus niger in glucose The data are summarized in table 8. Complete inhibibroth. tion of this mold was caused by the addition of 0.27 per cent acetic acid, 4.84 per cent lactic acid, or 0.35 per cent hydrochloric acid to glucose broth. The limiting pH values were 4.1, 2.2, and 1.3 respectively. In other words, less acetic than either lactic or hydrochloric acids was required to prevent mold growth and the toxic limit when acetic acid was used was at a lower hydrogenion activity than that of the other two acids. There was some inhibition as indicated by only a slight growth when acetic or hydrochloric acids were present in a concentration of 0.21 per cent. At non-toxic concentrations, the mold readily utilizes lactic acid to aid in the development of a heavy, rubbery mat unlike those produced in the presence of non-toxic amounts of either acetic or hydrochloric acids. Within non-toxic limits those flasks which contained the more lactic acid had the heavier mold growth. In the lactic acid series it is also interesting to note that acidities of 3.48 and 2.46 per cent gave rise to mats of approximately the same This is not surprising if the final acidities are deducted weight. from the initial acidities to determine the amounts of acid utilized. Thus, with an initial acidity of 3.48 per cent and a final acidity of 1.24 per cent, 2.24 per cent lactic acid was utilized to yield a dry weight of 495.7 mgm. When the initial acidity was 2.46 per cent and the final acidity 0.24 per cent, 2.22 per cent lactic acid was utilized to yield a dry weight of 486.3 mgm. These figures are in accordance with other initial acidities and final yields of mold in this series.

Again it is evident that the toxicity of acetic acid to mold spores is a function of the nature of the acid, perhaps in addition to the hydrogen-ion activity. In the case of lactic acid, too, the toxicity seems to be due to more than the hydrogen-ion activity alone as the limiting pH value was higher in the lactic acid series than the limiting pH value of the hydrochloric acid series. However, the toxic principle of the lactic acid was far less potent toward spores of A. niger than the toxic factor of the acetic acid.

DISCUSSION AND CONCLUSIONS

The premise is fairly well established that an increase in acidity or in hydrogen-ion concentration inhibits the growth of, or even destroys, certain microorganisms and materially reduces their resistance to heat. The results of this investigation dealing mainly with the activity of acetic acid on certain microorganisms related to food spoilage confirm the findings of other investigators and cast some light on a somewhat obscure phenomenon: the peculiar toxicity of acetic acid.

It has been demonstrated that acetic acid in small amounts and at relatively high pH values proved toxic to representative aerobic bacteria and a yeast and mold. Lactic and hydrochloric acids were toxic to these same test organisms but at a higher total acid concentration or at a lower pH. The lethal activity at an unquestionably higher pH value than that obtained with hydrochloric acid is an indication that the effect of acetic acid is not due to the hydrogen-ion activity alone. The toxicity of acetic acid cannot be attributed to its general organic nature and slight ionization because lactic acid in larger quantities was required to effect the same toxicity despite the fact that a higher hydrogen-ion activity was observed with lactic acid than with an equivalent amount of acetic acid. This latter point is mentioned because it is true that the toxicity is increased at higher hydrogen-ion concentrations.

The results, in general, show that the hydrogen-ion activity cannot be entirely responsible for the toxicity of acetic acid. Many investigators are of the opinion that the lethal effect of the toxic organic acids is due, at least in part, to the undissociated molecule. However, the mechanism of inhibition is mainly a matter of conjecture. Wynne (1931) states that it is the accumulation of adsorbed substance which seems to exert some influence on the cell, but one can only speculate as to the essential physiological effect of this concentrated layer of adsorbed substance, resulting in the failure of the cell to function normally. That the influence of adsorbed acid is due to something more than the mere physical presence of a foreign substance is proved by the fact that adsorption of the saturated paraffin series of fatty acids by bacteria is often possible without upsetting their metabolism.

Not only can acetic acid inhibit and destroy microorganisms when used in sufficiently high concentrations but it also aids materially in reducing the thermal death points of bacteria when present in sub-lethal concentrations. Many practical applications can be inferred from this phenomenon. The addition of a small amount of acid exerts more of a beneficial effect on the preservation of canned foods by lowering the thermal death point than by inhibiting the growth of the organism. Some of the major problems in the canning of foods are related to the high heat treatment necessary for the commercial sterilization of the so-called "non-acid" products. The addition of acetic acid is justified provided the appearance and flavor are not noticeably altered. The long accepted usage of vinegar as a common food component precludes any objection to this acid from a physiological standpoint.

SUMMARY

The toxic effect of vinegar upon certain microorganisms is usually attributed to its acetic acid content.

Acetic acid in nutrient broth inhibited the growth of various microorganisms related to food spoilage. The bacteria used did not grow in broth adjusted with acetic acid to pH 4.9. Saccharomyces cereviseae did not grow at pH 3.9 and Aspergillus niger was inhibited at pH 4.1.

An increase in the hydrogen-ions resulted in a decrease of the thermal death points of the bacteria studied. The reduction in lethal temperature was more marked in the case of *Bacillus mesentericus* and *Bacillus cereus* than with the non-spore forming organisms. Thermal death points of the yeast and the mold were unaltered by the addition of small amounts of acetic acid.

Comparative studies showed acetic acid to be more toxic than either lactic or hydrochloric acid to Salmonella aertrycke, Saccharomyces cereviseae, and Aspergillus niger. These organisms were inhibited or destroyed at a higher pH value with acetic acid than with lactic or hydrochloric acids. The mold utilized relatively high amounts of lactic acid to develop a growth heavier than that obtained from the acetic acid or the hydrochloric acid series.

Because of its lethal activity at comparatively high pH values, the toxicity of acetic acid for various microorganisms is not confined to the hydrogen-ion concentration alone but also seems to be a function of the undissociated acetic acid molecule.

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PLATE 1

Comparative Effects of Acetic, Hydrochloric, and Lactic Acids on the Growth of Aspergillus Niger

Left to right:	Per cent acidity
Control	0.03
Acetic acid	0.27
Acetic acid	0.21
Hydrochloric acid	0.30
Hydrochloric acid	0.12
Lactic acid	3.48
Lactic acid	1.80

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PLATE 1