ON THE EFFECT OF ACIDS, ALKALIS, AND NEUTRAL SALTS ON THE FERMENTATIVE ACTIVITY AND ON THE RATE OF MULTIPLICATION OF YEAST CELLS

By ERIC DRABBLE, D.Sc., F.L.S., AND DAISY G. SCOTT, M.Sc.

(Received May 2nd, 1907)

In the present communication we give an account of some observations on the effect of acids, alkalis, and neutral salts on the rate of fermentation induced by yeast cells, and also some notes on the rate of multiplication of the yeast cells in culture media to which a neutral salt has been added to increase the osmotic pressure.

That plants grow less rapidly in concentrated than in dilute solutions was first shown by Jarius (1). His experiments were conducted upon the germination of seeds. Eschenhagen (2), working with Fungi, showed that the maximum strength in which the plants will grow differs from species to species, but for any one species is of the same osmotic strength for different salts.

Raciborski (3) stated that in *Basidiobolus* the maximal concentration for growth in solutions of sodium chloride is that exercising an osmotic pressure of about seventeen atmospheres, i.e., 6 per cent.

Livingston (4 and 5) finds that the Alga *Stigeoclonium* fails to produce zoospores in strong salt solutions.

I. FERMENTATION IN THE PRESENCE OF ACIDS, ALKALIS, AND NEUTRAL SALTS

Solutions of sodium chloride, potassium chloride, sodium nitrate, potassium nitrate, hydrochloric acid, nitric acid, sodium hydroxide and potassium hydroxide were made up in strengths 0.1, 0.01, 0.001 and 0.0001 gram-molecular. One cubic centimetre of 50 per cent. solution of cane-sugar was made up to 26 c.c. with each of the solutions

of acid, alkali, and neutral salt named above. These were set up in duplicate in test tubes. To these were added yeast cells in such proportion that each tube, on a count being taken by means of the usual square-cell method, gave an average number of 26.5 cells per square. The dilution of the solution consequent on addition of sugar and yeast was calculated and allowed for, and the tubes were placed in the dark for a week. At the end of seven days the tubes were examined, and the state with regard to fermentation was noted. In the records given the note 'floating bubbles' represents a vigorous fermentation, while 'floating yeast' indicates the most active condition in this respect.

TABLE I

State of fermentative activity of Yeast in solutions of cane-sugar in various media after seven days.

Gram-molecular strength of medium			State of fermentative activity	
Sodium chloride	...	0.1	}	{ Fermentation, floating bubbles.
"	"	0.1		{ Fermentation, floating bubbles.
"	"	0.01	}	{ Much fermentation, floating yeast.
"	"	0.01		{ Fermentation, floating bubbles.
"	"	0.001	}	{ Much fermentation, floating yeast.
"	"	0.001		{ Much fermentation, floating yeast.
"	"	0.0001	}	{ Much fermentation, floating yeast.
"	"	0.0001		{ Fermentation, little floating yeast.
Potassium chloride	...	0.1	}	{ Fermentation, floating yeast.
"	"	0.1		{ Little fermentation, little floating yeast.
"	"	0.01	}	{ Fermentation, little floating yeast.
"	"	0.01		{ Little fermentation, little floating yeast.
"	"	0.001	}	{ Fermentation, very little floating yeast.
"	"	0.001		{ Little fermentation, some floating bubbles.
"	"	0.0001	}	{ Fermentation, little floating yeast.
"	"	0.0001		{ Little fermentation, little floating yeast.
Sodium nitrate	...	0.1	}	{ Fermentation, floating yeast.
"	"	0.1		{ Fermentation, floating yeast.
"	"	0.01	}	{ Fermentation, little floating yeast.
"	"	0.01		{ Fermentation, floating yeast.
"	"	0.001	}	{ Fermentation, little floating yeast.
"	"	0.001		{ Fermentation, floating yeast.
"	"	0.0001	}	{ Fermentation, floating yeast.
"	"	0.0001		{ Fermentation, floating yeast.

Potassium nitrate ...	O'I	}	{Some fermentation, floating yeast.
" " ...	O'I	}	{Some fermentation, little floating yeast
" " ...	O'OI	}	{Much fermentation, floating yeast.
" " ...	O'OI	}	{Fermentation, floating yeast.
" " ...	O'OOI	}	{Much fermentation, floating yeast.
" " ...	O'OOI	}	{Fermentation, floating yeast.
" " ...	O'OOOI	}	{Fermentation, floating yeast.
" " ...	O'OOOI	}	{Fermentation, floating yeast.
Hydrochloric acid ...	O'I	}	{No fermentation.
" " ...	O'I	}	{No fermentation.
" " ...	O'OI	}	{Very little fermentation, 3 floating bubbles.
" " ...	O'OI	}	{Very little fermentation, few floating bubbles.
" " ...	O'OOI	}	{Little fermentation, few floating bubbles.
" " ...	O'OOI	}	{Some fermentation, floating bubbles.
" " ...	O'OOOI	}	{Little fermentation, few floating bubbles.
" " ...	O'OOOI	}	{Fermentation, floating bubbles.
Nitric acid ...	O'I	}	{No fermentation.
" " ...	O'I	}	{No fermentation.
" " ...	O'OI	}	{Little fermentation, no floating bubbles.
" " ...	O'OI	}	{Very little fermentation, no floating bubbles.
" " ...	O'OOI	}	{Little fermentation, few floating bubbles.
" " ...	O'OOI	}	{Some fermentation, floating bubbles.
" " ...	O'OOOI	}	{Some fermentation, very little floating yeast.
" " ...	O'OOOI	}	{Some fermentation, very little floating yeast.
Sodium hydroxide ...	O'I	}	{No fermentation.
" " ...	O'I	}	{No fermentation.
" " ...	O'OI	}	{Some fermentation, trace of floating yeast.
" " ...	O'OI	}	{Little fermentation, floating bubbles.
" " ...	O'OOI	}	{Some fermentation, floating yeast.
" " ...	O'OOI	}	{Some fermentation, floating bubbles.
" " ...	O'OOOI	}	{Fermentation, floating yeast.
" " ...	O'OOOI	}	{Fermentation, floating yeast.
Potassium hydroxide ...	O'I	}	{No fermentation.
" " ...	O'I	}	{No fermentation.
" " ...	O'OI	}	{Some fermentation, floating yeast.
" " ...	O'OI	}	{Little fermentation, floating bubbles.
" " ...	O'OOI	}	{Fermentation, floating yeast.
" " ...	O'OOI	}	{Fermentation, floating yeast
" " ...	O'OOOI	}	{Fermentation, floating yeast.
" " ...	O'OOOI	}	{Fermentation, floating yeast.

A consideration of the results summarised in the preceding table shows clearly that none of the neutral salts employed in the media exercises a marked inhibiting action on the fermentative activity of the enzyme within the range of strengths used. Both hydrochloric and nitric acids in concentration 0·1 gram-molecular completely inhibit the fermentative action; in 0·01 gram-molecular concentration the yeast is still slightly active, while in 0·001 and 0·0001 the fermentation is progressively greater.

In the case of sodium and potassium hydroxides the case is generally similar; in 0·1 fermentation is completely inhibited. In 0·01 it is only slight, but is more pronounced than in the same concentration of an acid. This is doubtless partly due to the fact that the evolved carbon dioxide is absorbed by the solution of hydroxide, whereby the amount of this substance in solution is reduced. In 0·001 and 0·0001 the fermentation is considerably more active.

II. EFFECT OF THE SOLUTION ON THE YEAST CELLS

After seven days the state of the cells in the media containing hydrochloric acid, potassium hydroxide and potassium chloride was examined. When the cells were scattered and not budding the presumption is that they were inactive, a conclusion entirely supported by the state of fermentation in the tubes. When the cells instead of being uniformly scattered were found generally to be aggregated into clumps the activity was greater, while in the most active and healthy cells buds were being produced and active reproduction was going on.

A measurement of the mature cells in these tubes was made. The results of these observations are recorded in the following table :—

<i>Hydrochloric Acid</i>			
Strength in gram-molecules		Condition of the cells	Average diameter of the cells
0·1	...	Scattered, not clumped	7·3
0·01	...	Slightly clumped	9·4
0·001	...	Distinctly clumped	9·4
0·0001	...	Clumped and budding	9·4

<i>Potassium Hydroxide</i>				
Strength in gram-molecules		Condition of the cells		Average diameter of the cells
0·1	...	Scattered, not clumped	...	9·8
0·01	...	Slightly clumped, some buds	...	9·7
0·001	...	Much clumped and budding	...	9·7
0·0001	...	Much clumped and budding	...	9·7

<i>Potassium Chloride</i>				
0·1	...	Very much clumped, budding	...	9·5
0·01	...	Very much clumped, budding	...	9·8
0·001	...	Very much clumped, budding	...	9·9
0·0001	...	Very much clumped, budding	...	9·9

It will be noticed that in the hydrochloric acid tubes the cells are scattered, and, judging from their size, are killed at 0·1 gram-molecular strength. As has been shown elsewhere (6), yeast cells shrink when killed in acid. In the solutions of less strength the cells are clumped and are active, possessing a uniform average size in all the strengths. For potassium hydroxide the same holds true, only in 0·1 gram-molecular strength the cells are not reduced in size being, indeed, rather larger than in the weaker strengths.

In these lower strengths of alkali they possess a uniform average size rather greater than the size in the equivalent strengths of acid. In the neutral salt, potassium chloride, the cells are clumped and actively budding in each tube, but here it is interesting to note that the osmotic concentration of the solutions seems to come into play and results in progressively smaller-sized cells in 0·0001, 0·001, 0·01, and 0·1 gram-molecular solutions.

After seven days the yeast cells in the tubes were counted by means of the squared-cell method with the following results :—

The original number of cells per square was 26·5.

<i>Sodium Chloride</i>			<i>Potassium Chloride</i>		
0·1	gram-mol.	32·1	0·1	gram-mol.	35·8
0·01	"	37·3	0·01	"	35·7
0·001	"	45·7	0·001	"	33·7
0·0001	"	37·9	0·0001	"	36·2

<i>Sodium Nitrate</i>			<i>Potassium Nitrate</i>		
0·1	gram-mol.	31·6	0·1	gram-mol.	56·5
0·01	„	32·3	0·01	„	40·9
0·001	„	34·2	0·001	„	44·2
0·0001	„	32·7	0·0001	„	36·5

<i>Hydrochloric Acid</i>			<i>Nitric Acid</i>		
0·1	gram-mol.	24·5	0·1	gram-mol.	22·1
0·01	„	37·2	0·01	„	30·4
0·001	„	42·3	0·001	„	34·0
0·0001	„	44·8	0·0001	„	50·3

<i>Sodium Hydroxide</i>			<i>Potassium Hydroxide</i>		
0·1	gram-mol.	22·6	0·1	gram-mol.	25·5
0·01	„	29·4	0·01	„	26·6
0·001	„	42·1	0·001	„	33·2
0·0001	„	46·9	0·0001	„	46·7

This count of the cells yields some interesting figures. The original average number per square was 26·5. In all strengths of neutral salts an increase in the number of cells is manifest, as could have been predicted safely from the fact that they are actively budding in these tubes. In the acids of strength 0·1 gram-molecular, on the other hand, not only has multiplication failed to take place but an actual reduction has been brought about by a certain proportion of the cells having been killed. These killed cells are seen as transparent structures which have lost entirely their granular appearance and were not counted in estimating the numbers. In the other strengths of acid there has been an increase in number.

In the tubes of hydroxides again there has been an actual reduction in number of cells in 0·1 gram-molecular strength. In all the other tubes there has been an increase in number except in 0·01 potassium hydroxide where the number is approximately equal to that at the commencement of the experiment.

III. MULTIPLICATION OF YEAST CELLS IN SOLUTIONS OF SODIUM NITRATE

A complete series of tubes was set up in duplicate in solutions of sodium nitrate from 0.1 to 1.0 gram-molecular strength. One cubic centimetre of 50 per cent. cane sugar solution was added to each tube, and yeast to produce eighteen cells to each square was placed in the solution, due allowance being made for the dilution thus caused.

The following results were obtained after seven days in the dark :—

Strength of solution in gram-molecules		Osmotic pressure of solution in millimetres of mercury		Number of cells		State of fermentative activity
0.1	...	3018	...	29.5	...	Fermentation, floating bubbles. Fermentation, floating bubbles.
0.2	...	6067	...	32.8	...	Fermentation, floating bubbles. Fermentation, floating bubbles.
0.3	...	8948	...	21.0	...	Fermentation, floating bubbles. Fermentation, floating bubbles.
0.4	...	11762	...	20.0	...	Fermentation, floating bubbles. Fermentation, floating bubbles.
0.5	...	11490	...	19.5	...	Fermentation, floating bubbles. Fermentation, few floating bubbles.
0.6	...	17287	...	18.5	...	Little fermentation, 1 bubble. Little fermentation, 2 bubbles.
0.7	...	19829	...	18.2	...	Little fermentation, 2 bubbles. Little fermentation, 3 bubbles.
0.8	...	22541	...	17.6	...	No active fermentation, no bubbles. No active fermentation, no bubbles.
0.9	...	24021	...	16.7	...	No active fermentation, no bubbles. No active fermentation, no bubbles.
1.0	...	27625	...	14.8	...	No active fermentation, no bubbles. No active fermentation, no bubbles.

The following curve has been plotted to show graphically the number of cells in each tube :—

Curve showing relation between strength of solution of sodium nitrate and the number of yeast cells developed after 7 days. Ordinates express the number of yeast cells, abscissae the osmotic pressure of the solution in millimetres of mercury. Original number of yeast cells in each strength of solution was 18.

It is of interest to note that the most active production of new cells does not take place in the solution of least osmotic strength but in a solution of pressure equal to that of 6067 mm. of mercury. This strength agrees closely with that of the normal yeast juice as was shewn in a previous communication (6).

From that point onwards increase in osmotic strength of the solution results in a progressively decreasing rate of reproduction until in a solution of osmotic pressure equivalent to 19829 mm. of mercury the cells fail to reproduce at all. This is approximately the pressure at which the protoplasm ceases to exercise any pressure on the

cell-wall, as was shown in the communication to which reference has been made (6). In still greater concentrations of solution the yeast not only fails to reproduce but is actually killed, the number of cells being less than when the experiment was set up.

SUMMARY

1. The neutral salts, sodium and potassium chlorides and nitrates, fail to exercise any marked inhibiting action on the fermentative activity of yeast when added to sugar solution in strengths of 0.1, 0.01, 0.001 and 0.0001 gram-molecular.

2. Hydrochloric and nitric acids of 0.1 gram-molecular strength entirely prevent fermentation. In the weaker strengths they exercise depressing action.

3. Sodium and potassium hydroxides of 0.1 gram-molecular strength entirely prevent fermentation. The weaker solutions 0.01 and 0.001 depress the action, while 0.0001 affects the process but little.

4. Hydrochloric and nitric acids in strength 0.1 completely inhibit reproduction of the yeast and indeed are fatal to the cells in some degree. The lower concentrations permit reproduction of the cells.

5. In all strengths of the neutral salts employed the cells reproduce actively.

6. The hydroxides are to some extent lethal at a concentration of 0.1 gram-molecular and at this strength prevent any reproduction. In the lower concentrations reproduction takes place (except in 0.01 potassium hydroxide).

7. The effect on the average size of the cell in the case of 0.1 hydrochloric acid is very marked, shrinkage of the diameter to that of the dead cell taking place.

8. In a series of cultures containing sodium nitrate of all strengths differing by unity in the first place of decimals from 1.0 to 0.1 gram-molecular, the greatest rate of reproduction was found in 0.2. From this stage increasing strengths of the salt depressed

the reproductive activity down to 0·7, where no reproduction took place. In greater strengths the solutions proved lethal to some of the cells, so that a smaller number of cells was found at the end of the experiment than at the beginning.

LITERATURE

1. Jarius. 'Ueber d. Einwirkung von Salzlösungen auf d. Keimungsprocess d. Samen einiger einheimischer Culturgewächse' (*Landwirtsch. Versuchs Stat.*, Vol. XXXII, 1886).
2. Eschenhagen. 'Ueber den Einfluss von Lösungen verschiedener Konzentration auf das Wachsthum von Schimmelpilze,' Stolp, 1889.
3. Raciborski. 'Ueber den Einfluss äusserer Bedingungen auf die Wachstumsweise d. *Basidiobolus ranarum*,' *Flora*, Vol. LXXXII, 1896.
4. Livingston. 'On the nature of the stimulus which causes change of form in polymorphic Green Algae,' *Bot. Gazette*, Vol. XXX, 1900.
5. Livingston. 'Further notes on the Physiology of Polymorphism in Green Algae,' *Bot. Gazette*, Vol. XXXII, 1901.
6. Drabble (E. and H.) and Scott. 'On the size of the cells of *Pleurococcus* and *Saccharomyces* in solutions of a neutral salt.' *Bio-Chem. Journ.*, Vol. II, pp. 221-229.