CXXXVI. SELECTIVE FERMENTATION.
III. FERMENTATION OF HEXOSE-PENTOSE MIXTURES.

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The mutual influence of fermentable hexoses on the fermentation of each has been studied using several varieties of yeast and has been reported in preceding communications [Sobotka and Reiner, 1930, 2, 3]. In the present study, we have investigated the influence of non-fermentable sugars on the fermentation of fermentable hexoses. We measured the rate of carbon dioxide formation from mixtures of glucose or fructose with a pentose and also determined in one series of experiments the total amount of carbon dioxide developed from such mixtures.

It appears that the fermentation by brewer's yeast of glucose, and more so that of fructose, is retarded in the presence of xylose and also of arabinose. This effect was observed in all sugar concentrations tested from 0·8 to 6·7 % and with yeast concentrations from 0·6 to 15 %, under aerobic and anaerobic conditions. The inhibitory effect of the pentose varied parallel with the ratio of its concentration to that of the hexose, leading sometimes to almost complete suppression of fermentation, particularly with yeasts that had been kept more than 24 hours in the ice-chest. Fructose fermentation was inhibited more than glucose fermentation, and xylose proved a stronger inhibitor than arabinose.

While fermentation went slower in the presence of the non-fermentable pentoses, it progressed further. Thus, the deficit between the actual and the theoretical amounts of carbon dioxide obtained at the end of fermentation was decreased in the presence of xylose, even vanishing in some instances.

In analogous experiments with baker's yeast no such effects on the rate and amount of fermentation could be observed. On the contrary, in the case of aerobic fermentation of glucose by baker's yeast an acceleration of the development of carbon dioxide took place when xylose was present.

In an attempt to explain these observations the possibility that the total sugar concentration reached a level high enough to impair the progress of fermentation could be eliminated. On seeking more specific explanations, competition of the pentose with the hexose in one of the early stages of fermentation had to be considered. Concerning phosphorylation no disappearance of inorganic phosphate could be observed with xylose when using a dry yeast preparation which effectively phosphorylated glucose in parallel experiments.

In order to evaluate the possible influence of diffusion on the "pentose-effect", a study was undertaken of the rate at which various sugars diffuse into the yeast cell. To this end known amounts of yeast were treated with a solution of a given concentration of xylose, arabinose or other unfermentable carbohydrate. Samples of the supernatant, withdrawn at intervals, revealed the rate of disappearance of the sugar from the solution into the yeast cell. This rate of diffusion is rarely high enough to allow an equilibrium to be established. With most of the sugars tested, diffusion was so slow, that no equilibrium was reached

within 18 hours, at which time the yeast had begun to autolyse. But the amount of sugar diffused within a given initial period, *e.g.* one hour, serves well for purposes of comparison. In order to make the diffusion rate of fermentable sugars available for comparison, fermentation had to be suppressed. This was accomplished by means of iodoacetic acid in a dilution sufficient to prevent fermentation but without significant influence on the rate of diffusion as tested with xylose.

According to the speed of diffusion into the yeast cell the compounds tested may be arranged in the following order: xylose > glucose > galactose > *d*-arabinose > *l*-arabinose > mannitol > rhamnose > lactose. The remarkable speed with which xylose is taken up by the cell suggests that this sugar competes with the fermentable hexoses so effectively for entrance into the yeast cell that it may retard their fermentation under certain conditions. The diffusion rates of fructose and glucose were compared in one experiment (no. 85) using yeast that had been treated with iodoacetic acid. The diffusion of fructose was about one-fourth slower than that of glucose, and this difference may be responsible (*a*) for the faster fermentation of glucose from glucose-fructose mixtures (previous papers), (*b*) for the greater inhibition of fructose than of glucose fermentation in the presence of xylose and (*c*) for the moderate inhibitory effect of arabinose on fructose fermentation. The slower diffusion of arabinose as compared with xylose explains why only the latter has a significant effect on glucose fermentation. It has been shown [Nord and Weichherz, 1929] that diffusion is a controlling factor in fermentation, since rapid stirring of fermenting sugar solutions may accelerate the rate of fermentation. Thus, it is likely that competitive diffusion is one of the causes of the "pentose-effect". However, it should be noted that the diffusion measurements yielded analogous results for baker's and for brewer's yeast, but that the pentose inhibition was confined to brewer's yeast.

The great differences in rate of diffusion of various sugars are of interest beyond the explanation of the mechanism of the "pentose-effect" and of selective fermentation. They demonstrate that in the domain of diffusion phenomena small structural and steric differences can cause as high a degree of specificity as one is accustomed to associate with enzymic and immunological reactions. Rate of diffusion is not determined by a simple physical property such as molecular weight or merely by molecular structure, but depends also on steric configuration. Thus, it differs for two substances whose properties are identical except for their opposite optical activities as in the case of *d*- and *l*-arabinose. This confirms our belief that diffusion is based on transient chemical combination with constituents of the cell-wall. The comparison of the diffusion of xylose, arabinose, glucose and galactose shows an analogy between the pair xylose-glucose and arabinose-galactose in regard to the speed of diffusion. As the structural resemblance in each pair issues from the aldehydic group, it can be assumed that this group enters into the chemical reactions with the cell-wall constituents responsible for the selective diffusion phenomena in our experiments.

The study of diffusion of non-fermentable sugars into unicellular organisms can be applied to the differentiation of extracellular, intracellular and chemically bound water in pressed yeast and similar products. At most 90 % of the water contained in yeast, as determined by drying at about 100°, is accessible as solvent for xylose or other sugars entering the cell, and for the less rapidly diffusing sugars this limit is never reached. 10 to 15 % of the water is chemically bound in the cell constituents, especially the plasma protein, and is not available as solvent. On the other hand, with a sugar like lactose which practically does not diffuse into the yeast cell, one may observe an instantaneous disappearance of a few per cent. from the supernatant fluid indicating the free and immediate accessi-

bility of about one-tenth of the water content of the pressed yeast. This is the amount of moisture on the surface of the cells and in the spaces between them and corresponds to the figures for extracellular water obtained by rapid washing with chilled alcohol or acetone. This amount of extracellular water is subject to great variations depending on the degree of pressure used on the yeast, and these variations are reflected in the variations of lactose absorption (*e.g.* Exps. 74 and 75).

The increased yield of carbon dioxide from hexose fermentation in the presence of pentose must be attributed to a hexose-saving effect of the pentose. Whether the pentoses enter any of the side-reactions such as glycerol formation, which are made responsible for the usual carbon dioxide deficit in alcoholic fermentation, or play some part in respiration and formation of polysaccharides and other cell-constituents, by replacing hexoses in other reactions, they release them for alcoholic fermentation. It is likely that such sugar-saving effects of non-fermentable carbohydrates contained in molasses are utilised in industrial fermentations without recognition of the underlying mechanism.

Experimental.

Fermentation of mixtures of a hexose with a pentose.

The experiments recorded in Table I were carried out in the Van Iterson-Kluyver apparatus [Sobotka and Reiner, 1930, 2, p. 928]. The amounts of sugar and of fresh yeast and the volume of solution, which was always pre-saturated

Table I. *Fermentation of mixtures of a hexose with a pentose.*

No.	Hexose mg.	Pentose mg.	Yeast g.	Vol. ml.	Time for half fermentation: Without pentose min.	Time for half fermentation: With pentose min.	Ratio of Rate with pentose / Rate without pentose %	Yield of CO_2: Without pentose % of theory	Yield of CO_2: With pentose % of theory
				Lager yeast					
1	Glucose 50	Xylose 50	0·2	2·0	81	91	89	—	—
	„ 50	Arabinose 50	0·2	2·0	—	84	96	—	—
	Fructose 50	Xylose 50	0·2	2·0	84	103	81	—	—
	„ 50	Arabinose 50	0·2	2 0	—	98	86	—	—
2	Glucose 50	Xylose 25	0·2	1·5	121	153	79	—	—
	„ 50	Arabinose 25	0·2	1·5	—	135	90	—	—
3	Glucose 50	Xylose 50	0·2	2·0	36	48	75	71	89
	Fructose 50	„ 50	0·2	—	42	53	79	64	76
4	Glucose 30	Xylose 30	0·2	1·3	25	27	93	86	92
	„ 60	„ 60	0·2	1·6	46	51	90	86	88
5	Glucose 30	Xylose 30	0·2	1·3	19	29	65	—	—
	„ 60	„ 60	0·2	1·6	34	40	85	—	—
6	Glucose 50	—	0·2	1·5	47	—	—	93	—
	„ 50	Xylose 100	0·4	3·0	24	30	80	91	97
	„ 50	„ 300	0·8	6·0	<21	23	<90	80	96
7	Fructose 50	—	0·2	1·5	76	—	—	101	—
	„ 50	Xylose 100	0·4	3·0	42	54	78	89	103
	„ 50	„ 300	0·8	6·0	29	46	63	97	107
				Baker's yeast					
8	Glucose 50	Xylose 50	0·2	1·0	67	68	98	85	87
	Fructose 50	„ 50	0·2	1·0	71	77	92	86	87
9	Glucose 50	Xylose 100	0·2	1·0	81	80	101	87	87
	Fructose 50	„ 100	0·2	1·0	85	92	92	84	85

Table II. *Rate of fermentation in absence and presence of xylose.*

All fermentations carried out in Barcroft-Warburg manometers at 30° with 16 mg. yeast (*ca.* 4 mg. dry weight) in 2 ml.

No.	Glucose %	Xylose %	μl. CO_2 developed in 30 min. Without xylose	With xylose	Ratio of Rate with pentose / Rate without pentose — % Pentose / Hexose 1:1	2:1	3:1	Remarks
				Lager yeast				
10	5·0	5·0	488	548	112	—	—	—
	5·0	5·0	447	456	102	—	—	Anaerobic
11	5·0	5·0	364	333	92	—	—	—
	5·0	5·0	529	445	84	—	—	Anaerobic
12	6·7	6·7	250	199	80	—	—	—
13	5·0	5·0	523	377	72	—	—	—
14*a*	2·5	2·5	137	130	95	—	—	—
	—	5·0		105	—	77	—	—
15*a*	2·5	2·5	225	214	95	—	—	—
	—	5·0		189	—	84	—	—
16*a*	2·5	2·5	142	116	82	—	—	Anaerobic
	—	5·0		100	—	71	—	,,
17*a*	2·5	7·5	67	39	—	—	58	,,
18*a*	2·5	7·5	331	158	—	—	48	—
					92	77	53	Averages
				Baker's yeast				
19	2·5	5·0	116	218	—	188	—	—
	2·5	5·0	569	601	—	106	—	Anaerobic
20	2·5	5·0	180	273	—	151	—	—
21*a*	2·5	5·0	184	236	—	128	—	—
22*a*	2·5	5·0	187	220	—	118	—	—
23*a*	2·5	5·0	580	599	—	103	—	Anaerobic initial
	2·5	5·0	536	514	—	96	—	Anaerobic after 240 min.
	Fructose %							
				Lager yeast				
14*b*	2·5	2·5	143	130	91	—	—	—
	2·5	5·0	150	125	83	—	—	—
15*b*	2·5	2·5	197	182	92	—	—	—
	—	5·0	277	209	75	—	—	—
24	5·0	5·0	365	301	83	—	—	—
25	2·5	2·5	254	205	81	—	—	—
26	5·0	5·0	464	353	76	—	—	—
27	2·5	3·75	157	117	—	75	—	Pent.:Hex. = 1·5:1
28	2·5	5·0	189	16	—	8	—	Initial
	2·5	5·0	301	63	—	20	—	After 170 min.
	2·5	5·0	278	118	—	42	—	After 280 min.
29*a*	2·5	5·0	397	369	—	93	—	—
30	2·5	6·25	130	71	—	—	55	Pent.:Hex. = 2·5:1
29*b*	2·5	7·5	397	315	—	—	79	—
31	2·5	7·5	288	7	—	—	3	—
16*b*	2·5	2·5	129	122	96	—	—	Anaerobic
	2·5	5·0	125	108	—	86	—	,,
17*b*	2·5	7·5	44	19	—	—	43	,,
18*b*	2·5	7·5	325	200	—	—	62	,,
					86	71	48	Averages
				Baker's yeast				
21*b*	2·5	5·0	199	225	—	113	—	—
22*b*	2·5	5·0	192	209	—	109	—	—
23*b*	2·5	5·0	610	588	—	96	—	Anaerobic

Experiments numbered 14*a* and 14*b*, etc. were carried out simultaneously using samples of the same yeast.

with carbon dioxide, are given in Table I. The theoretical amount of carbon dioxide obtainable from the given amount of fermentable sugar was calculated and corrected for temperature (22° to 27°). The gas burettes were shaken and read every five minutes. The rate of fermentation is expressed by the time in minutes required for half fermentation, *i.e.* for the development of 50 % of the amount of carbon dioxide theoretically possible [Sobotka, 1924]. The samples of baker's yeast used in this and in the following series displayed uniformity in their fermenting power. Greater deviations in rate of fermentation under identical conditions were experienced with the various specimens of brewer's yeast depending on freshness, season and other factors beyond our control. The total solids varied from 25 to 28 %.

The series of experiments recorded in Table II was carried out in the Barcroft-Warburg apparatus, using conical vessels of about 20 ml. capacity in a water-bath kept at 30·0°. The amount of yeast applied was invariably 16 mg., the total volume 2 ml. The velocities given in Table II are initial velocities and are expressed in μl. carbon dioxide developed during the first 30 min. In some instances, the observations were interrupted after this period, but the fermentation was allowed to continue with the stopcocks opened; readings were then resumed after 2 or 5 hours. At this time, because of the relatively small amount of yeast, the sugar content was far from exhausted and fermentation still proceeded at full speed, the absolute and relative rates not deviating from the initial rates to a significant degree (see Exp. 23*a*), except in Exp. 28, where the fermentation in presence of pentose was almost completely suppressed in the beginning, but increased in the subsequent stages. Regardless of the variations in fermenting power of the Lager yeast, xylose consistently exerted a retarding influence. This effect remained unaltered under anaerobic conditions. On the other hand, in the case of baker's yeast no retardation was observed, but in the fermentation of glucose the rate of carbon dioxide development was frequently accelerated.

Diffusion experiments.[1]

Approximately 5 % solutions of the various sugars were prepared and the exact concentration was determined. Fresh yeast was washed twice with distilled water to remove any reducing substances, centrifuged at high speed and pressed. 20 g. of the pressed yeast were then suspended by thorough mixing in 20 ml. of the sugar solution. The amount of sugar varied between 120 and 180 mg. per g. of dry yeast except in Exps. 62*b*, 62*c*, and 70. After the intervals given in Table III aliquot samples of the suspension were withdrawn and centrifuged, and the clear supernatant was analysed for sugar by Hanes's modification of the Hagedorn-Jensen method, using the tables of Sobotka and Reiner [1930, 1]. Mannitol was determined polarimetrically after saturation of the solution with borax. The amount of sugar which had passed into the yeast was calculated from the original amount *minus* the residual sugar content of the supernatant.

The water content of the yeast determined by drying at 100° was made the basis of the calculation of how much sugar could be removed from the supernatant if the dissolved sugar reached the same concentration within as without the cell; *e.g.* when 20 g. of yeast containing 28·7 % total solids (5·74 g.) are added to 20 ml. of sugar solution, the amount of potential solvent is increased to 34·26 ml. In case of an ideal equilibrium 14·26/34·26 parts or 41·7 % of the

[1] A preliminary report on these studies was given at the December Meeting 1933 of the Biochemical Society, London.

Table III. *Diffusion of various sugars into the yeast cell.*

20 ml. of approximately 5% sugar solutions were mixed with 20 g. yeast (total solids 2 to 3%). From these data we calculated in each instance the amount of sugar that would disappear from the supernatant if equilibrium of the sugar concentration within and without the cell were established. The figures given in the table are the percentages of this amount which have actually disappeared from the supernatant at a given time.

	Sugar	Diffusion after 15 min.	1 hr.	2 hrs.	18 hrs.	
		Experiments with brewer's yeast (Lager)				
51	Xylose	88·4	—	—	—	After 5 min.
52	Galactose	69·6	—	84·5	—	—
53*a*	*d*-Arabinose	44·3	73·2	—	—	—
53*b*	*l*-Arabinose	33·9	44·4	—	—	—
54*a*	*d*-Arabinose	31·1	67·2	—	—	—
54*b*	*l*-Arabinose	25·8	38·7	—	—	—
55	Rhamnose	20·4	31·2	—	—	—
56	Mannitol	30·2	—	—	—	—
57*a*	α-Lactose	14·6	20·7	—	—	—
57*b*	β-Lactose	16·5	22·7	—	—	—
58	Lactose	13·4	22·4	—	—	Courtesy Milk Sugar Institute
59	Lactose	15·8	—	—	—	—
		Experiments with baker's yeast (Fleischmann)				
60	Xylose	76·5	—	—	—	After 5 min.
61	Xylose	83·8	—	—	—	,,
62*a*	Xylose	82·8	—	—	—	—
62*b*	Xylose	80·2	—	—	—	From a 10% xylose solution
62*c*	Xylose	75·9	—	—	—	From a 20% xylose solution
63	Glucose	61·0	—	—	—	After 5 min.
64	Glucose	95·4	—	—	—	Beginning fermentation?
65	Maltose	51·1	—	—	—	28·9% after 5 min.
66	Galactose	26·0	—	67·7	—	—
67	Galactose	—	24·9	65·8	81·7	—
68	*d*-Arabinose	18·7	—	31·0	—	—
69*a*	*d*-Arabinose	—	38·1	67·7	91·1	—
69*b*	*dl*-Arabinose	—	29·9	55·2	88·4	—
69*c*	*l*-Arabinose	—	22·1	24·3	52·3	—
70	*dl*-Arabinose	—	69·8	73·6	94·3	From 2·5% arabinose solution
71	Rhamnose	24·2	33·0	—	—	—
72	Mannitol	—	33·3	—	—	—
73	Mannitol	17·0	23·2	—	—	—
74	Lactose	9·6	20·3	—	—	—
75	Lactose	—	—	9·1	—	Extra dry yeast (total solids 34%)
		Baker's yeast treated with iodoacetic acid 0·4%				
76	Xylose	83·2	—	—	—	—
77	Xylose	71·2	82·0	—	—	—
78	Xylose	69·7	—	—	—	—
79	Xylose	67·5	77·2	—	—	—
80	Xylose	—	88·5	88·5	90·8	—
81	Glucose	61·3	—	—	—	—
82	Glucose	44·7	53·4	—	—	—
83	Glucose	44·1	—	—	—	—
84	Glucose	34·8	56·7	—	—	—
85*a*	Glucose	—	45·4	55·1	81·2	—
85*b*	Invert sugar	—	56·9	61·1	85·9	—
85*c*	Fructose	—	34·0	42·5	62·0	—
		Experiments with *Saccharomyces marxianus*				
86	Maltose	67·0	—	—	—	—
87*a*	Glucose	51·8	—	—	—	Fermentation suppressed with 0·4% CH_2ICOOH
87*b*	Maltose	32·8	—	—	—	,,

dissolved sugar, *e.g.* 401 mg. out of 963 mg. would disappear from the supernatant, provided that under the conditions of the experiment the volume of the yeast cells remains constant; this was ascertained in control experiments. As an additional check, the yeast, after having taken up a known amount of a non-fermentable sugar, was separated and re-suspended in a given amount of distilled water: thus, we could demonstrate that the sugar would diffuse from within the cell to the outside in the expected measure when the gradient of concentration was reversed.

The yeast samples used in Exps. 76–85, and 87, were treated for 15 min. with an equal amount of an aqueous 0·4 % solution of iodoacetic acid, which was sufficient to suppress any subsequent fermentation.

The inability of some yeasts to ferment certain carbohydrates, and on the other hand, the adaptation of some strains of yeast to unaccustomed sugars remain to be investigated from the viewpoint of selective diffusion. The inability of *Saccharomyces marxianus* to ferment maltose [Sobotka and Reiner, 1930, 2] is due to other factors, as diffusion of maltose in this yeast occurred at normal speed (Exp. 86), more than half of the rate of glucose diffusion (Exp. 87, *cf.* Exps. 63 and 65).

Selective diffusion. In a few instances an attempt was made to compare the rates of diffusion of two components of a sugar mixture. In experiments on the simultaneous diffusion of *d*- and *l*-arabinose and of glucose and fructose it was found that the *l*-(+)arabinose diffuses half as rapidly only as its *d*-(−)isomeride into the yeast. In the case of invert sugar, the glucose molecules penetrate the cell wall at about 1·33 times the rate of those of fructose. Table IV illustrates the

Table IV. *Diffusion of sugar mixtures.*

Time hrs.	Outside solution		Outside solution sugar partition %		Intracellular sugar partition (calculated) %	
	% sugar	$[\alpha]_D$	*l*-Arabinose	*d*-Arabinose	*l*-Arabinose	*d*-Arabinose
No. 69*b*						
0	5·28	0	50·0	50·0	—	—
1	4·64	+ 5·2°	52·5	47·5	31·2	68·8
2	4·10	+ 7·3	53·5	46·5	38·1	61·9
18	3·40	+ 3·5	51·7	48·3	46·8	53·2
No. 70						
0	2·59	0	50·0	50·0	—	—
1	1·80	+13·3	56·3	43·7	36·1	63·9
2	1·76	+19·3	59·2	40·8	30·7	69·3
18	1·54	0	50·0	50·0	50·0	50·0
No. 85*b*			Fructose	Glucose	Fructose	Glucose
0	4·62	−19·0	49·5	50·5	—	—
1	3·52	−19·9	50·1	49·9	47·6	52·4
2	3·44	−22·8	52·1	47·9	41·9	58·1
18	2·96	−18·0	49·0	51·0	50·4	49·6

manner of calculation of the relative speeds of diffusion in Exps. 69*b*, 70, and 85*b*, where the supernatant was analysed polarimetrically for the individual components in addition to the determination of the total sugar content.

Summary.

1. Pentoses, particularly xylose, have a retarding effect on the rate of fermentation of hexoses by brewer's yeast; however they sometimes increase the total amount of CO_2 evolved.

2. The rates of diffusion into the yeast cell of a number of fermentable and non-fermentable sugars differ to a considerable and significant degree. Competitive diffusion is a major factor in the mechanism of the "pentose-effect" and of selective fermentation.

3. Studies of diffusion permit the differentiation of extracellular, intracellular and chemically bound water in yeast.

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REFERENCES.

Nord and Weichherz (1929). *Chem. Ztg.* **53**, 393.
Sobotka (1924). *Z. physiol. Chem.* **134**, 1.
—— and Reiner (1930, 1). *Biochem. J.* **24**, 394.
—— —— (1930, 2). *Biochem. J.* **24**, 926.
—— —— (1930, 3). *Biochem. J.* **24**, 1783.