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Effect of the Nitrogen Content of Cells on Fermentation by *Aerobacter*

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While studying the dependence of the enzymatic activity of cells on their nitrogen content [3] we also turned our attention to the fermentation processes [1]. De Ley [2] observed with *Aerobacter aerogenes* K_3* that anaerobic fermentation activity rapidly lessened with the decrease in the nitrogen content of the cells and ceased entirely when the nitrogen content was lowered from the normal of about 13 % to approximately 8 % of dry matter. Respiration as well as aerobic acid formation is then still strong. Thus, by lowering the N-content of cells it is possible to change their metabolism in a very remarkable way. Our experiments with the same bacterial strain have also shown that fermentation is retarded when the nitrogen content of cells is lowered, though not as much as in the experiments of De Ley. However, a bacterial mass containing 9.5 % nitrogen had, in our experiments, a fermentation velocity (calculated per unit N of the cells) of only half that of the normal-N cellmass containing 13 % nitrogen (*cf.* below). Virtanen and Aejmelaeus [3] found with *Torula utilis*, too, that the rate of fermentation was lowered by 50 % when the N-content of the cells fell from 9.8 % to 5 % of dry matter. By adding β-alanine to the aerated *Torula* suspension in sugar solution it was possible to lower the N-content to 3.7 %. This low-N Torula still fermented glucose very slowly (activity about 20 % of the normal). Respiration and catalase activity of *Torula* increased considerably up to a nitrogen content of 4.5–5 %. Virtanen and De Ley [4] have dealt with the dependence of the enzymatic activity of cells on their N-content.

* The strain used by Virtanen and De Ley, taken from the laboratory collections labelled as *Escherichia coli* K_3 proved to be of aerogenes type.

In the present paper we shall record results of the experiments which were made in order to examine to what extent the N-content of cells affects the fermentation products and hence the fermentation mechanism. These results are still in some respects incomplete but even as such they indicate the distinct effect of the N-content of cells on the fermentation products of glucose in *Aerobacter*.

EXPERIMENTAL

Preparation of bacterial material. *Aerobacter aerogenes* strain K_3 was cultured parallel in two nutrient solutions, the one containing 8 g $(NH_4)_2SO_4$, the other 0.1 g $(NH_4)_2SO_4$. The composition was otherwise the same, *i.e.:* 25 g. K_2HPO_4, 10 g NaCl, 0.1 $MgSO_4$, and 25 g saccharose in 5 litres of tap water. The cultures were kept at 33° C for 65 hours and the acidity of the culture, to which was added bromothylmolblue as an indicator, was maintained at pH about 6.5 by adding, when necessary, 4 *N* NaOH-solution during the growth. The growth was far better on the greater amount of ammonium sulphate than on the smaller. The bacteria were separated by centrifugation and washed with sterile water. The bacterial mass was suspended in 50 ml of sterile water as homogeneously as possible and for each fermentation experiment was used either 10 ml suspension of the normal-N bacteria or 30 ml of the low-N bacteria. The N-content of each suspension was determined.

Fermentation experiment. Fermentation solution: 50 ml of m/8 phosphate buffer (pH 6.1) with 250 mg glucose. 0.2 ml of 1 % water solution of bromcresolpurple was added. The pH was maintained throughout the fermentation at about 6.0—6.2 by adding alkali as required. The fermentation bottle had a wide mouth closed with a rubber stopper. The stopper was furnished with two glass tubes, the one serving as outlet for the evolved gases which were collected in a mercury sealed gas burette, the other as inlet for the 0.1 *N* NaOH used for neutralisation of the solution. Before the start of fermentation oxygen was removed from the system by a current of nitrogen. Temperature of the thermostat was 37° C. The bottles were shaken mechanically during the fermentation, gas readings being made at intervals of half an hour.

In two experiments sugar was estimated according to Bertrand at intervals of 2 hours. The determinations were made from bottles, which were not connected with the gas burette.

Analytical methods. The fermentation solution was centrifuged and analyzed for acetic acid and formic acid according to Virtanen and Pulkki [5], for formic acid separately according to Riesser [6], for lactic acid according to the principle of Führt-Charnass [7] and for alcohol according to Sémichon and Flanzy [8]. Succinic acid was determined according to Tasman and Smith [9]. With solutions containing lactic acid in amounts corresponding to our fermentation solutions, the method gave in check analyses values which were about 8 % too high. The values obtained were therefore corrected respectively. The total volume of gas evolved was determined volumetrically but CO_2 and H_2 were not determined separately.

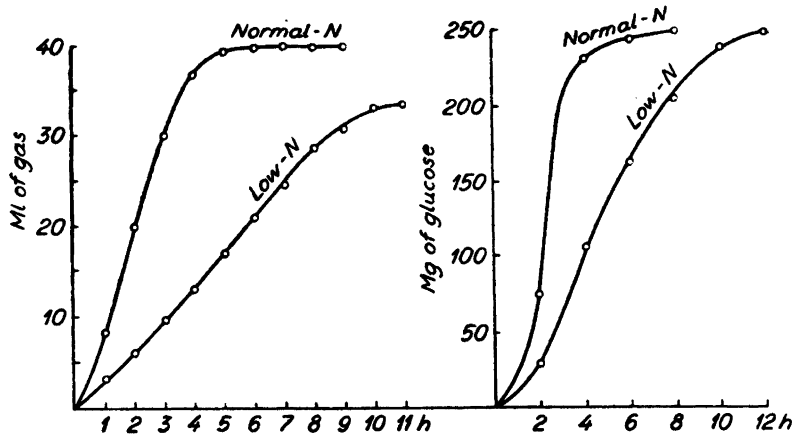

Fig. 1. Fermentation of 250 mg glucose in parallel experiments by normal-N and low-N masses of Aerobacter aerogenes K_3. Gas formation (left) and consumption of sugar (right).

RESULTS AND DISCUSSION

Fermentation velocity was followed in experiment 6 by gas evolution and decrease of sugar (Fig. 1). The low-N bacterial mass used in this experiment and the normal-N bacterial mass contained respectively 9.5 % and 13.1 % N based on dry matter. Judged both by gas evolution and sugar consumption, the rate of fermentation with normal-N mass was about twice as high as with the low-N mass, when calculated per unit nitrogen of the bacterial suspensions used. On the other hand, in experiment 5, in which the low-N bacterial mass contained still 10.5 % N of dry matter, the fermentation velocity was not considerably lower than with normal-N mass.

The fermentation products of normal-N and low-N bacterial masses are shown in Table 1.

Table. 1. Fermentation products of normal-N and low-N bacterial masses in per cent of fermented glucose

Expt. No.	N % of dry bacteria		Formic acid		Acetic acid		Lactic acid		Succinic acid		Ethanol	
	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N
2	13.0	9.3	4.5	5.4	2.4	17.5	42	22	29	35	11	8
3	12.3	9.4	6.5	5.0	4.0	10.8	39	21	22	40	—	—
4	12.8	9.4	6.5	5.5	1.5	13.9	40	21	29	40	12	15
5	13.1	9.5	5.6	4.6	2.7	10.5	42	21	28	38	12	11
6	13.2	10.5	6.0	6.5	2.0	6.6	42	29	26	31	11	14

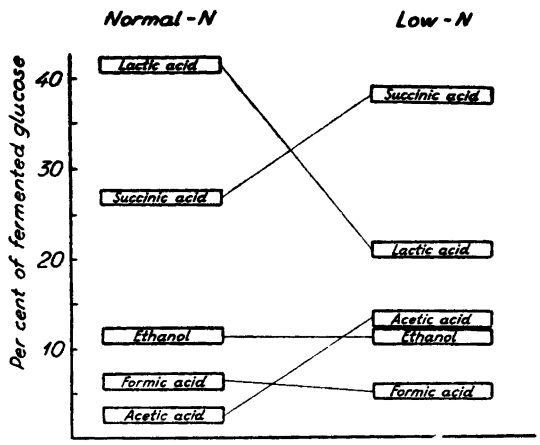

Fig. 2. Graphical illustration of the mutual proportions of different fermentation products with normal-N (avg. 12.8 %) and low-N (avg. 9.4 %) bacterial masses. Experiments 2, 3, 4, and 6 are included.

The results show that *Aerobacter* masses, which contain about 9.3—9.5 % N of dry matter, form from glucose only about half as much lactic acid as do the normal-N *Aerobacter*. These same low-N masses formed 3—9 times more acetic acid than did the normal-N masses, and about 30 % more succinic acid. In the production of formic acid and ethanol no systematic differences were noted. Results from experiment 5, in which the low-N bacterial mass contained more nitrogen, will be discussed below.

Graphical illustration in Fig. 2 shows the differences in the fermentation products formed from glucose by normal-N and low-N bacterial masses.

Since all the conditions in the parallel experiments with low-N and normal-N bacterial masses were as similar as possible, the differences in the fermentation products are probably due to differences in the enzyme system of these bacterial masses. Because of the small amount of lactic acid formed by low-N bacteria it would seem likely that the activity of lacticodehydrogenase has lessened with the decrease in the protein content of cells and therefore the reduction of pyruvic acid to lactic acid is retarded. This, again, would explain the increase in the decomposition of pyruvic acid via another route and the increase in the acetic acid and succinic acid.

It is interesting to note that Tikka [10] in this laboratory found as early as in the 1930's that fermentation products of *E. coli* essentially depend on the acidity of the fermentation solution. At about pH 6.3 the production of lactic acid was about 40 % and that of acetic acid about 5 %, whereas at pH 7.1

the amount of each was about 20 %. The quantitative differences observed by us between the fermentation products of normal-N and low-N *Aerobacters* are very much alike although the pH of both fermentation solutions was kept as constant as possible, *i.e.* 6—6.2. Lowering of the N-content of cells thus affects the enzyme system functioning in the fermentation perhaps in the same way as does the change of pH from acid to alkaline in the fermentation solutions of normal-N bacteria.

Additional evidence that retardation of fermentation and change in the mutual relations of the fermentation products are in fact due to the decrease in the N-content of cells, is provided by the results obtained with low-N *Aerogenes* bacteria of different N-content. Expt. 5 in Table 1 represents fermentation products of a bacterial mass, which contained 10.5 % N of dry matter. The fermentation velocity with this mass was nearly the same as with normal-N masses and the fermentation products differed much less from those obtained with normal-N masses than from those obtained with masses containing 9.3—9.5 % N.

The carbon content of the analyzed fermentation products expressed as per cent of the carbon in glucose is given in Table 2.

Table 2. Carbon content of the analyzed fermentation products in per cent of carbon in glucose acc. to Expt. 6

	C in per cent of C in glucose	
	Normal-N bacteria	Low-N bacteria
Formic acid	3.6	3.0
Acetic acid	2.7	10.5
Lactic acid	41.8	21.0
Succinic acid	28.3	37.0
Ethanol	16.4	14.3
	92.8	85.8

The carbon dioxide content of the gas evolved was not determined, hence, the carbon thus removed is unknown. However, it is evident that normal-N bacteria have not formed considerable amounts of other fermentation products except those mentioned above. On the other hand, low-N bacteria have formed other products in quite appreciable quantities, perhaps acetylmethylcarbinol and butyleneglycol. However, they were not determined and hence, no fermentation balance can be presented as a result of the analyses.

SUMMARY

Low-N *Aerobacter aerogenes* ferments glucose at a noticeably slower rate than onrmal-N bacteria. If the N-content of the cells is lowered from normal, by about

13 %, to 9.3—9.5 % of dry matter the fermentation velocity decreases by about half. Lowering of the N-content to 10.5 % affects but slightly the velocity.

The mutual proportions of the fermentation products are greatly changed by the decrease in the N-content. The cells containing 9.3—9.5 % N produce only about half as much lactic acid as do the normal-N cells, but much more acetic acid and succinic acid. No systematic differences were observed in the amounts of ethanol and formic acid. Low-N bacteria probably form significant amounts of other fermentation products, too, which have not been determined. The results are discussed.

REFERENCES

1. Virtanen, A. I. *120th Meeting Am. Chem. Soc., New York, N. Y. Sept. 3 to 7, 1951, Abstracts of Papers* 2 A.
2. De Ley, J. *Verhandel. Koninkl. Vlaam. Acad. Geneesk. Belg.* **11** (1949) no. 3. Doctoral thesis.
3. cf. Virtanen, A. I. *Colloid. Chem.* **7** (1950) 255.
4. Virtanen, A. I., and De Ley, J. *Arch. Biochem.* **16** (1948) 169.
5. Virtanen, A. I., and Pulkki, L. *J. Am. Chem. Soc.* **50** (1928) 3147.
6. Riesser, O. *Z. physiol. Chem.* **96** (1916) 355.
7. Lieb, H. and Zacherl. M. K. *Z. physiol. Chem.* **211** (1932) 211.
8. cf. Winton, A. L., and K. B. *The Analysis of Foods.* (1945). New York.
9. Tasman, H., and Smith, L. *Rec. trav. chim.* **67** (1948) 404.
10. Tikka, J. *Ann. Acad. Sci. Fennicae, Ser. A.* **41** (1935) no. 4; *Biochem. Z.* **279** (1935) 264, 288.

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