

INFLUENCE OF NITRATES, NITRITES AND POLYPHOSPHATES ON BREATHING
ACTIVITY OF STR. FAECALIS

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Summary,

Studies are carried out on the influence of NaNO_3 , NaNO_2 and $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ as used in meat processing, upon the breathing activity of *Str. faecalis*. Demonstrated is, that nitrates and polyphosphates act in some cases as inhibitors, while in others as stimulants for glucosis oxydation from *Str. faecalis*, in direct relation with the used concentrations. Nitrites, independant of their concentration, demonstrate only inhibitory action.

The inhibitory action of nitrites and the specified concentrations of nitrates and polyphosphates is exhibited strongly while studying *Str. faecalis* cells in their logarithmic phase of growth.

These results, permit some valuable for the production considerations.

**ДЕЙСТВИЕ НИТРАТОВ, НИТРИТОВ И ПОЛИФОСФАТОВ НА ДЫХАТЕЛЬНУЮ
АКТИВНОСТЬ КЛЕТОК STR. FAECALIS**

Резюме

Исследовано влияние NaNO_3 , NaNO_2 , $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ в концентрациях, используемых в производстве мясных продуктов, на дыхательную активность клеток *Str. faecalis*.

Установлено, что нитраты и полифосфаты действуют в одном случае как ингибиторы, а в другом как стимуляторы, в зависимости от их концентрации, при окислении глюкозы клетками *Str. faecalis*.

Нитриты, независимо от их концентрации, проявляют только ингибирующее действие.

Ингибирующее действие нитритов и определенных концентраций нитратов и полифосфатов проявляется более сильно при исследовании клеток *Str. faecalis*, находящихся в логарифмической фазе роста.

Полученные результаты позволяют сделать важные для производства выводы.

**DIE EINWIRKUNG VON NITRATEN, NITRITEN UND POLYPHOSPHATEN
AUF DIE ATMUNGSAKTIVITÄT BEI STR. FAECALIS**

Zusammenfassung

Untersucht wurde die Einwirkung von NaNO_3 , NaNO_2 , $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ in den üblichen Konzentrationen bei der Fleischverarbeitung auf die Atmungsaktivität bei *Str. faecalis*. Abhängig von der verwendeten Konzentration wirkten die Nitrat und Polyposphosphate hemmend oder stimulierend auf die Oxydation der Glykose durch *Str. faecalis* ein.

Die Nitrite aber unabhängig von ihrer Konzentration hatten immer nur einen hemmenden Effekt.

Die hemmende Einwirkung der Nitrite und der bestimmten Konzentration der Nitrat und Polyposphosphate ist am stärksten bei den untersuchten Zellen, welche sich in der logarithmischen Phase der Entwicklung befinden.

Daraus lässt sich wichtige Schlussfolgerung für die Herstellung von Fleischwaren ziehen.

Glycolysis which represents a complex of anaerobic enzymatic processes of glucose degradation has a great biological significance in the vital activity of microorganisms. The way in which glucose

degradation is carried out may determine also the presence of other components. Particularly the presence of high concentrations of phosphorus in the medium inhibits glucose-6-phosphate dehydrogenase activity. At that, glucose degradation is carried out by way of anaerobic glycolysis, since instead of 6-phosphogluconate, fructose-6-phosphate is formed under the action of phosphohexoisomerase (7, 8, 9).

Unlimited phosphate slows down also the further decomposition of riboso-5-phosphate. Riboso-5-phosphate utilization rate is inversely proportional to ortho-phosphate concentration in the medium. High ortho-phosphate concentrations inhibit the formation of pyruvic acid from 2-phosphoglyceric acid. The phosphorus concentrations inhibiting the reactions indicated are not absolute quantities. With the increase of carbohydrate concentrations in the medium, the optimum phosphorus concentration is also increased, respectively (1).

In the study of the oxidative activity of *E. coli* cells with regard to soluble substrates and the different intermediate products of Krebs' cycle it was demonstrated that with bacteria, oxygen uptake in the oxidation of NAD.H, NADP.H, succinate, pyruvate and α -ketoglutarate is not stimulated by the addition of ATP and phosphate (3).

The rate of activation or inhibition of glucose oxidation by phosphate depends not only on its concentration in the medium, but also on its chemical nature. Inorganic phosphate is usually present in the solution in several forms (H_3PO_4 , $H_2PO_4^{1-}$, HPO_4^{2-} , PO_4^{3-}), some of which are involved into the reaction with enzymatic systems in the synthesis of ATP (2). Authors who have studied the action of pyridoxine phosphates on the growth of *Str. faecalis*, have proved that pyridoxine-5-phosphate is 16 times as active as pyridoxine. This action is considerably weaker than the effect exerted on the culture by pyridoxal, pyridoxamine, pyridoxal phosphate and pyridoxamine phosphate (7).

In the literature available to us we did not find any data about the action of $NaNO_3$ and $NaNO_2$ on the process of substrate oxidation by microorganisms. Presumably the character of the action of nitrates and nitrites depends on their concentrations in the medium, and also on the species of microorganism studied. So for example, the ratio between the CO_2 formed and the O_2 used (RQ) did not change after the exogenous addition of $NaNO_3$ to a culture of *Str. faecalis*. This ratio in *S. oryzae* did not change either

after the exogenous addition of NaNO_3 , regardless of the fact that O_2 utilization and CO_2 formation have increased about twice in this case (5).

The objective of the present investigations is the study of the effect of nitrates, nitrites and polyphosphates, in concentrations applied in meat processing, on the breathing activity of *Str. faecalis*.

METHODS

A suspension of 6 hr. and 16 hr. cultures of *Str. faecalis* var. liquefaciens strain 755 was used in the investigations. Bacteria were separated from the liquid medium (4) by way of centrifugation and were then washed twice with physiological solution (6).

The breathing activity was determined using Warburg's apparatus, in atmospheric air for 60 min. at 28°C ($\pm 0,1^\circ\text{C}$). Oxygen consumption by the cells was determined manometrically, and breathing (Q) was expressed in mm^3 of O_2 per 1 hour, related to 1 mg of cellular carbon. The incubation medium for 1 ml of suspension had the following composition: phosphate buffer, 1,0 ml (pH 7,4); glucose, 0,5 ml (0,6% solution); NaNO_3 , NaNO_2 or $\text{Na}_4\text{P}_2\text{O}_7$, 0,5 ml (0,025, 0,08 and 0,25% sol. for NaNO_3 ; 0,0125, and 0,125% sol. for NaNO_2 ; and 0,2, 0,3 and 0,4% sol. for $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{ H}_2\text{O}$, respectively). The total volume of Warburg's vessel was 3,2 ml.

The breathing of *Str. faecalis* cells was determined parallelly in 2 vessels, each experiment being carried out at least 4 times.

RESULTS AND DISCUSSION

The results from the study of the effect of NaNO_3 on the breathing activity of *Str. faecalis* var. liquefaciens strain 755 are shown in Table 1.

Table 1. Effect of NaNO_3 on the consumption of O_2 ($\text{mm}^3/\text{mg}/\text{hour}$) by *Str. faecalis* cells during different growth periods

NaNO_3 , %	6 hours				16 hours		
	Amount of O_2 (mm 3 /mg/hour)	Inhibition, %	Stimulation, %	Amount of O_2 (mm 3 /mg/hour)	Inhibition, %	Stimulation, %	Amount of O_2 (mm 3 /mg/hour)
1	2	3	4	5	6	7	
Control	85,25	0	0	17,94	0	0	
Experiment, 0,025	88,98	-	4,37	18,67	-	4,07	

Table 1. (Continued)

1	2	3	4	5	6	7
Experiment, 0,08	79,27	7,02	-	17,03	5,10	-
Experiment, 0,25	81,43	4,48	-	17,54	2,25	-

The data in the table indicate, that NaNO_3 in low concentrations (0,025%) is a weak stimulator of the breathing of 6- and 16 hr. cultures of *Str. faecalis*. With higher concentrations of NaNO_3 , applied in meat processing (0,08% and 0,25%), a contrary slightly inhibitory effect is observed, which is better pronounced at 0,08% and decreases with the increase of concentration. This is observed regardless of the age of cells.

The stimulating or inhibitory effect of NaNO_3 which makes it-self felt, depending on the concentration, is better pronounced in 6 hr. cultures during the active logarithmic phase, than in 16 hr. cultures during the initial stationary phase. These results demonstrate that young *Str. faecalis* cells are more susceptible to the action of NaNO_3 , while mature cells exhibit a better resistance.

Table 2. Effect of NaNO_2 on the consumption of O_2 ($\text{mm}^3/\text{mg}/\text{hour}$) by *Str. faecalis* cells during different growth periods

NaNO_2 , %	6 hours			16 hours		
	Amount of O_2 consumed ($\text{mm}^3/\text{mg}/\text{hour}$)	Inhibition, %	Stimulation, %	Amount of O_2 consumed ($\text{mm}^3/\text{mg}/\text{hour}$)	Inhibition, %	Stimulation, %
Control	85,25	0	0	17,94	0	0
Experiment, 0,0125	77,97	8,54	-	17,13	4,53	-
Experiment, 0,125	70,43	17,59	-	16,56	7,68	-

In Table 2, the data about the effect of NaNO_2 on the breathing activity of *Str. faecalis* var. liquefaciens strain 755 are shown.

In contrast to NaNO_3 , the results indicate that, in the concentrations applied in meat processing, NaNO_2 exerts only an inhib-

bitory action which increases with the increase of the concentration. Here also the inhibitory effect is better pronounced in young 6 hr. cultures during the active logarithmic growth phase.

Table 3. Effect of $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$ on the consumption of O_2 ($\text{mm}^3/\text{mg}/\text{hour}$) by Str. faecalis cells during different growth periods

$\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$, %	6 hours			16 hours		
	Amount of O_2 ($\text{mm}^3/\text{mg}/\text{hour}$) consumed	Inhibition of breathing, %	Stimulation of breathing, %	Amount of O_2 ($\text{mm}^3/\text{mg}/\text{hour}$) consumed	Inhibition of breathing, %	Stimulation of breathing, %
Control	85,25	0	0	17,94	0	0
Experiment, 0,2	87,86	-	3,06	18,45	-	2,83
Experiment, 0,3	82,51	3,21	-	17,87	0,38	-
Experiment, 0,4	80,35	5,75	-	17,41	2,98	-

The effect of $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$ on the breathing activity of Str. faecalis var. liquefaciens strain 755 is shown in Table 3. The results are analogical to those with NaNO_3 . In low concentrations (0,2%), $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$ exhibits a weak stimulating action in the breathing of Str. faecalis. The higher concentrations (0,3% and 0,4%) applied in meat processing have an inhibitory effect on the breathing activity of Str. faecalis. Unlike NaNO_3 , with the increase of the concentration the inhibitory effect of $\text{Na}_4\text{P}_2\text{O}_7$ increases and is better pronounced in the 6 hr. cultures.

On comparison, the data from Tables 1, 2 and 3 indicate the prevailing inhibitory action of NaNO_3 , NaNO_2 and $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$, which is best expressed in NaNO_2 during the active logarithmic phase of the growth of Str. faecalis. The most active stimulating effect is observed in the 0,025% concentration of NaNO_3 .

These results have an important significance in the curing and canning of meat products.

CONCLUSIONS

1. NaNO_3 in low concentrations used in meat processing (0,025%), is a weak stimulator, and in higher concentrations (0,08 and

0,25%) it is a weak inhibitor of the exogenous breathing of Str. faecalis.

2. The inhibitory effect of NaNO_3 on the breathing activity of Str. faecalis is better expressed at 0,08 % and decreases with the increase of the concentration.

3. The stimulating or inhibitory effect of NaNO_3 on the breathing activity of Str. faecalis is better pronounced in the young 6 hr. cultures in the active logarithmic growth phase, and it is weaker in the 16 hr. cultures which are in the initial stationary phase.

4. NaNO_2 in concentrations applied in the curing and production of meat items exhibits only an inhibitory action which increases with the increase of its concentration.

5. The inhibitory effect of NaNO_2 on the breathing activity of Str. faecalis is better expressed with cultures in the active logarithmic phase and weakens in the initial stationary growth phase.

6. $\text{Na}_4\text{P}_2\text{O}_7$ in low concentrations (0,2%) has a slight stimulating action on the exogenous breathing of Str. faecalis, and with the increase of concentrations (0,3%, 0,4%) it exerts an inhibitory action, which increases in the higher concentrations.

7. The stimulating or inhibitory effect of $\text{Na}_4\text{P}_2\text{O}_7$ on the breathing activity of Str. faecalis is more pronounced in 6 hr. cultures, and less so in 16 hr. cultures.

8. The stimulating and inhibitory effects of the different concentrations of NaNO_3 , NaNO_2 and $\text{Na}_4\text{P}_2\text{O}_7$ applied in meat processing on the exogenous breathing activity of Str. faecalis have an important significance in the curing and canning of meat products and exert an influence on their shelf life .

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