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On the Biodynamics of Starter Cultures

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The production of high quality dried meat products depends exclusively on the growth of the useful microflora during the technological process. Many authors (4,5,6,7,8,9,14,17,18,19, 20,22,23,26,27,28,29,30,33,35,36,37,38,40,42) prove the decisive part played by micrococcus and lactobacillus bacteria in the formation of the wanted colour, flavour and taste in ripening of meat products. These results gave grounds during the last years in many countries, the studies for the isolation, investigation, selection and use of micrococci and lactic acid bacteria to be widened and the microorganisms accepted as starter cultures in meat processing (17, 18, 20, 21, 34, 35, 42).

The development of the starter cultures in the raw dried meat products depends to a great extend on their sensitivity to NaCl, $NaNO_3$, $NaNO_2$, sugars, polyphosphates, lactic and ascorbic acids, contained in the product (1,2,3,5,9,10,11,12,13,16, 15, 19,23,24,25,26,31,32,33,39,43,47,48).

Since there are no data about the dynamics of development of the starter cultures, their respiratory activity, the growth of the biomass, protein syntheses in the separate growth phases and the influence of the preserving staining and binding substances on the activity of their respiration, during 1971 , we made a number of investigations in these directions (46).

In the present work we made our scope to elucidate those problems pertaining to the biodynamics of the starter cultures in connection with some bioenergetic and biosynthetic processes in their development.

METHODICS

The studies were made with culture of micrococcus strain 199/10 and lactic acid starter culture strain 4669/10, isloated in the Finnish Institute for Meat Technology.

The cultivation was made in Ehrlenmeyer flask containing 750 ml nutritive medium for micrococcus strain 199/10 with pH 7,2, containing 10 g of pepton (Merck), 3 g yeast and 10 g meat extracts (Oxoid); 5 g NaCl and 5 g glucose, diluted in 1 l water, and for the lactic acid culture strain 4669/10, liquid nutritive medium with pH 7,0, containing 10 g of pepton (Merck), 1,5 g yeast and 10 g meat extracts (Oxoid); 5 g NaCl and 5 g glucose, disolved in 1 l water.

For innoculum we used 24 hours broth culture, obtained at 37°C with continuous shaking. The microorganisms we separated from the medium by centrifugation and double washing with physiological serum. The obtained microbial mass we suspended in physiological serum and in this status used per innoculum of nutritive medium in quantity of $7,5.10^4$ microbial cells in 1 ml.

The samples were taken from the moment of the innoculation in the nutritive medium each other hour to the 48th, and centrifugated them with double washing with 100 ml physiological serum. The obtained biomass was used for all our investigations.

The growth of the biomass during the separate phases of development was determined after the colorimetricmethod, used by us with the enterococci, based on the quantitative determination of cell carbon (45).

The respiratory activity of both strains we determined on resting cells after the momometric methods of Warburg (49). In each Warburg vessel we put 1 ml resting cells suspension, 1,5 ml phosphate puffer (pH 7,4); in the inner vessels we indtroduced 0,2 ml 20% KOH solution and small cuts of filter paper which keep the potassium hydrate during the shaking. When the endogenic cell respiration was determined by way of the side entrance of the Warburg vessel we introduced 0,5 ml phosphate puffer; when the exsogenic respiration was studied, through the side entrance of the Warburg vessel we introduced 0,5 ml of 0,6% glucose solution, corresponding dilution to 3,2 ml (total volume of liquid in the vessel) or the content in meat products. Incubation temperature is 28 ±0,1°C in air. The oxygen used for 1 hour by the starter cultures during the separate growth phases we calculated to 1 mg cell carbon.

Protein content in the cells during the different phases was determined after the modified method of Lawry (41).

In the investigations on the oxidative possibilities of both starter cultures we used substrates, which diluted in the Warburg vessel, correspond to the following concentrations: glucose - 0,1%, lactic acid - 0,5% and ascorbic acid - 0,02% (corresponding to the content in meat products).

In determining the respiratory activity in the presence of NaCl, $NaNO_3$, $NaNO_2$, and $Na_4P_2O_7 \cdot 10H_2O$ we introduced these solutions through the side entrance in quantities of 0,5 ml with calculated concentrations, corresponding when diluted to 3,2 ml (total volume of the liquid in the vessel) quantity contained in meat products as follows: for NaCl 1,8; 2,2; 2,5; 3,0; 3,2; 3,5; 3,8; 4,0; 4,8; 5,6; 6,4 and 7% for $NaNO_3$ - 0,025%; 0,08% and 0,25%, for $NaNO_2$ - 0,0125 and 0,125% and for $Na_4P_2O_7 \cdot 10H_2O$ - 0,2; 0,3 and 0,4%.

The model solution represented a combination of the investigated substrates, having the same concentrations and inhibiting effect in relation and quantity as found in meat products: glucose 0,1%; lactic acid - 0,5%; ascorbic acid - 0,02%; NaCl - 1,8%; $NaNO_3$ - 0,025%; $NaNO_2$ - 0,0125% and $Na_4P_2O_7 \cdot 10\ H_2^{\circ}$ - 0,3.

The respiration of the two cultures we determined paralelly in two neigbour vessels, while each experiment was repeated at least four times.

RESULTS AND DISCUSSION

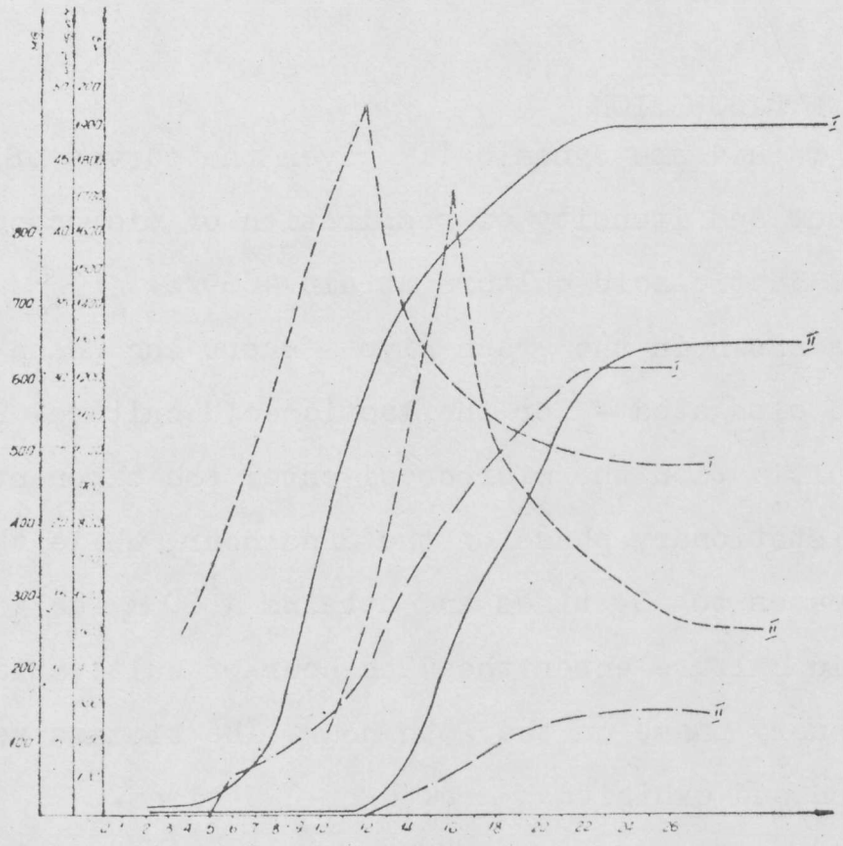
On graph 1 are dynamically given the curves of growth, protein content and itensity of respiration of micrococcus strain 199/10 and lactic acid culture strain 4669/6.

Data shown in the graph give a short lag phase for the micrococcus and elongated - for the lactic acid culture. On the 5th hour of cultivation the micrococci enter the exponential phase and pass into stationary phase on the 22nd hour, while the total biomass increases to 164 times and attains 1880 mg cell carbon. The lactic acid culture enter the 12th hour of cultivation passing into the stationary phase on the 26th hour. The biomass reaches 1300 mg cell carbon and exhibits a growth of 225 times.

Characteristic peculiarity of the micrococcus ist the short lag phase and the long exponental phase (18 hours) in which phase is observed a very energetic growth of the biomass from the 6th to the 12th hour, afterwards the growth is regulated and ends on the 24th hour. With the lactic a cid culture, differently from the micrococcus, a prolonged lag culture, differently from the micrococcus, a prolonged lag phase is observed of 12 hours and a long exponential phase of 14 hours running evenly and energetically. These peculiarities in the growth dynamics of the micrococcus and

GRAPH 1

Correlation between the respiratory activity, biomass growth and protein content during the different phases of development of micrococcus strain 199/10 and lactic acid starter culture strain 4669/6



I - Micrococcus strain 199/10
II - Lactic acid culture strain 4669/6

——————— biomass growth

-.-.-.-.- protein content

— — — — — respiratory intensivity

the lactic acid culture confirme their fitness for use as starter cultures alone or in combination. The dynamics of growth exhibits that the micrococcus has an active role in the first period of ripening of raw dried meat products and ist growth continues to the end of the process, while the lactic acid culture takes part primarily during the second period of ripening.

To elucidate the respiratory activity for both starter cultures in connection with the biomass growht, we studied and determined the intensivity of oxygen use from resting cells during the different phases of growth. The results given in graph 1 show for both cultures more intensive oxygen use in the inital and active exponential phase, than et the end of the exponential and the beginning of the stationary phases. The quantity of the used oxygen for the micrococcus is increased from 21,0 mm^3/mg/hr at the beginning of the exponental phase to 47,42 mm^3/mg/hr during the active exponential phase (on the 12th hour) and decreases to 24,0 mm^3/mg/hr during the stationary phase of development. For the lactic acid culture the used oxygen increases from 2.42 mm^3/mg/hr in the lag phase to 41,63 mm^3/mg/hour in the active exponential phase (16th hour) and decreases to 13,5 mm^3/mg/hr in the stationafy phase of development.These data indicate that the intensity of the exogenic respiration is related to the age and physiological condition of the cells and its maximum for the micrococcus is on the 12th hour, and for the lactic acid culture on the 16th hour, which coincides with their active exponential phase independant from the length of the lag phase.

A negligeable activity is observed for the endogenic respiration for both cultures, which for the micrococcus is within the limits of 0,8 to 3,6 mm^3/mg/hr, and for the lactic acid culture from 0,6 to 1,4 mm^3/mg/hr and these parameters are in close relation with the age of the cells.

Along with the biomass growth and respiratory activity we

investigated the dynamics of the biosynthesis of protein in the cells for both starter cultures.On graph 1 is seen, that in the lag phase total protein content for the micrococcus is stable (0,4 to 0,5 mg).With the transition into the initial exponential phase of growth (6th hour) a jump is observed to 53 mg followed by gradual increase of the protein content to the amount of 180 mg (12th hour).After that the quantity of the total protein shar ply increases to 360 mg and on the 22nd hour attains the maximal figure of 612 mg.For the lactic acid culture, the protein content does not change to the 12th hour (0,15 mg).After this a gradual increase is observed and on the 16th hour the protein byosynthesis is acceleratedtwice.In the stationary phase with this strain the prot tein contenton the biomass attains 140 mg.The sharp increase of the quantity of the protein in the biomass for the micrococcus after the 12th hour and for the lactic ac id culture after the 16th hour coincides with the momentum of their active exponential phase of development and the most intensive endogenic respiration in both cultures an corelates in dynamics with characteristic changes in the bioenergetic and biosynthetic activity of the mic robial cells.

To determin the activity of respiration with substrates glucose,lactic and ascorbic acids and a model solution we made investigations with both starter cultures, the result of which are given on graph 2.

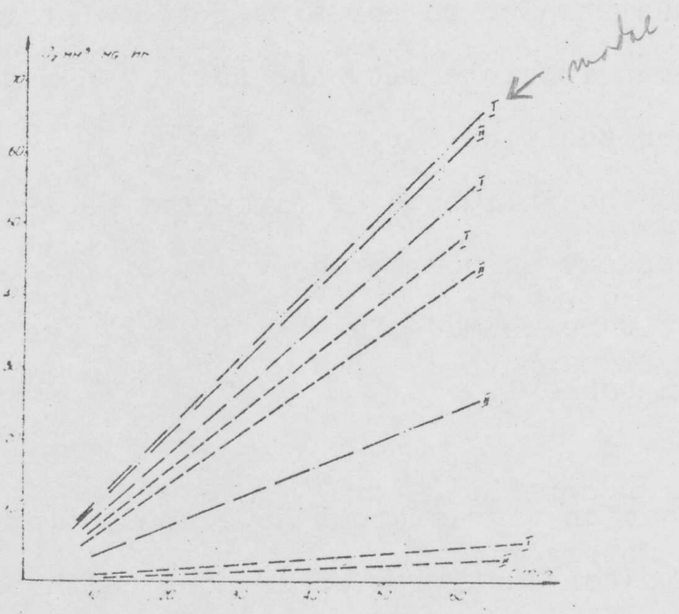
The investigated results shown in the graph speak for differences in the acceleration of the exydation of the separate substrates for both strain.

For the micrococcus is observed highest acceleration of oxydation with the model solution, followed by the lactic acid and glucose.The ascorbic acid for this strain is exibited as weak substrate in the exogenic respiration. Its action could be

accepted sooner as stimulating the endogenic respiration.

Dynamics of the oxydative property of microccocus strain 199/10 and lactic acid culture strain 4669/6 in connection with different substrates.

Graph 2



I - Microccocus strain 199/10 -•••-•••- model solution
II - L.acid culture strain ——————— glucose
4669/6 -•-•-•-•- lactic acid
--•--•--• ascorbic acid

For the lactic acid culture highest oxydation acceleration is observed for the model solution followed by glucose and lactic acid. Here too is observed negligeable use of the ascorbic acid in the exsogenic respiration.

In both strains a linear correlation is established between the quantity used oxygen and time for oxydation, independatly of used substrate.

The produced during the ripening lactic acid serves as sub strate for respiration of both cultures and could take part in the creation of favourable condition s for their growth.

The oxydative property of both cultures with the model solutions is near to that when lactic acid and glucose are used, which could be explained with the presence of inhibitor substances.

In both strains, the ascorbic acid acts as a weak substrate for the exsogenic respiration, which give us grounds to accept thaton practice the strater cultures do not desintegrate it, and this in turn ensures its integrity and action in meat products as a stabilizer for their colour.

To elucidate the influence of different NaCl concentrations on the intensity of oxygen use by the cells of microccocus and the lactic acid culture we made investigations, the results of which are given in table 1.

Table 1.

Inhibiting action of different NaCl concentrations on the respiratory activity of 12 hour cultures from microccocus and 16 hoyrs cultures of the lactic ac id cultures with th use of glucose, lactic and ascorbic acids.

Concentrations NaCl in %	% of respiratory depression					
	glucose		lactic acid		ascorbic acid	
	strain 199/10	strain 4669/6	strain 199/10	strain 4669/6	strain 199/10	strain 4669/6
Exper. 1,8	4,78	8,26	1,04	3,82	100,o	100,o
2,2	5,14	10,12	1,92	5,28	100,o	100,o
2,5	6,02	11,96	2,60	6,08	100,o	100,o
2,8	6,78	12,84	3,28	6,82	100,o	100,o
3,0	8,24	14,02	4,01	7,64	100,o	100,o
3,2	10,82	16,24	4,72	8,56	100,o	100,o
3,5	12,65	19,14	5,68	12,48	100,o	100,o
3,8	18,84	22,95	6,49	15,32	100,o	100,o
4,0	19,72	26,54	7,36	17,28	100,o	100,o
4,8	24,1	30,13	10,02	20,96	100,o	100,o
5,6	26,9	36,18	13,48	24,08	100,o	100,o
6,4	30,11	46,52	16,23	27,25	100,o	100,o
7,2	35,48	55,66	19,39	30,09	100,o	100,o
Control	0	0	0	0	100,o	100,o

Data shows that the added to the suspension cells from both strains, concentrations of NaCl (from 1,8 to 7,2%) exibit well defined inhibitor action on the oxygen use, while the percent of the depression on the respiration for all concentrations is higher for the lactic acid culture with the substrates glucose and lactic acid; Tha action of NaCl on the oxydative property of the cell from both cultures is most exibited with the use of ascorbic acid as substrate, the oxydation of which was attained 100% in comparison with the control with the addition of practically lowest possible concentrations of NaCl (1,8). Weakest inhibitor action exibits NaCl on the oxydation of the lactic acid, with the microccocus the percent of the respiratory depression of NaCl in concentration 7,2% is only 19,36%, and for the lactic acid culture it is 30,09%.

Parallel with this, we studied the influence of NaCl on the use of oxygen during the exponential and stationary phases of development of both cultures (table 2).

Table 2.

Influence of NaCl on the use of oxygen $mm^3/mg/hr$ of cells of microccocus strain 199/10 and lactic acid cultur strain 4669/6 in their exponential and stationary phases of development.

NaCl concentration in %	In exponential phase				In stationary phase			
	Quantity used oxygen O_2 in $mm^3/mg/hr$		Respiratory depression in %		Quantity used oxygen O_2 in $mm^3/mg/hr$		Respiratory depression in %	
	STRAIN 199/10	STRAIN 4669/6	STRAIN 199/10	STRAIN 4669/6	STRAIN 199/10	STRAIN 4669/6	STRAIN 199/10	STRAIN 4669/6
Exper. 1,8	45.15	38.19	4,78	8,26	24,00	13.39	0.00	0.78
2,2	44,98	37.42	5.14	10.12	23.90	13.33	0.42	1.26.
2.5	44.57	36.65	6.02	11.96	23.81	13.22	0.80	2.04
2,8	44.20	36.28	6.78	12.84	23.75	13.06	1.04	3.28
3.0	43.61	35.79	8.24	14.02	23.67	12.83	1.38	4.96
3.2	42.29	34.87	10.82	16.24	23.49	12.73	2.12	5.72
3.5	4141	33.66	12.65	19.14	23.07	12.55	3.86	7.02
3.8	39.43	32.08	16.84	22.95	23.01	12.46	4.12	7.74
4.o	38.04	30.58	19.72	26.54	22.79	12.39	5.03	8.24
4.8	35.99	29.09	24.10	30.13	22.58	12.15	5.92	10.02
5.6	34.67	26.57	26.90	36.18	22.37	11.94	5.78	11.53

6.4	33.14	22.26	30.11	46.52	22.21	11.90	7.46	11.83
7.2	30.60	18.46	35.48	55.66	22.02	11.83	8.26	12.34
Control	47.42	41.63	0.00	0.00	24.00	13.50	0.00	0.00

The above data show that the degree of respiratory depression of the cells in both strains, from the NaCl in the exponential and stationary phases of development is different.Strongest inhibitor action the NaCl exibits during the exponential phase. These data prove, that the young growing cells,in their process of active division (active exponential phase) are significantly more susceptive to the inhibitor effect of NaCl, while with age their sensitivity decreases.

To establish the influence of $NaNO_3$,$NaNO_2$ and $Na_4P_2O_7$ on oxygen use in cells from both strains starter cultures, in their exponential and stationary phases of development, we have made experiments, data of which are given in table 3.

With the micrococcus $NaNO_3$ in concentrations of 0.08 and 0.25% and $NaNO_2$ in concentrations of 0.0125 and 0.125% are weak stimulators of respiration for 12 and 48 hour cultures. The stimulating effect of $NaNO_3$ and $NaNO_2$ is exibited in connection with their concentrations, and is expressed in a weaker form for the $NaNO_2$ in the active exponential phase (for 12 hour cultures) than during the stationary phase (48 hour cultures).

With the lactic acid starter culture, the stimulating effect of $NaNO_3$ and $NaNO_2$ is expressed also in connection of their concentrations and respective development phases.This effect is expressed the strongest for 0.125% solution of $NaNO_2$ in the active exponential phase of development.

The polyphosphates in concentrations as used in the practice in meat processing exibit only an inhibitor action for both strains,which is increased with the increase of the respective concentration, and is stronger with the young, in their active division cells (in the active exponential phase of development).

Compared, data from tables 1,2,and 3 exibit a predominant inhibitor action of NaCl and $Na_4P_2O_7$, which for the NaCl is very distinctly shown in the active exponential phase of growth for both strains with the use of ascorbic acid and glucose as sub strates.

Most active stimulating action is observed for $NaNO_2$ in the concentration 0,125 for both strains.

These result have important significance in the use of starter cultures in meat processing in connection with the pro cesses of salting, preserving and ripening of the raw dried meat products.

C o n c l u s i o n s

1.Development dynamics for starter cultures is characte rised as follows: for microccocus strain 199/10 with a short lag phase (4 hours),a long exponential phase (18 hours) and a stationary phase after the 22 nd hour of growth, for the lac tic acid culture strain 4669/6 with an elongated lag phase (12 hours) long exponential phase (14 hours) and a stationary phase after the 26th hour of cultivation.

2.Growth dynamics of the investigated cultures show that the microccocus takes an active part in the first ripening pe riod of the raw dried meat products and its development conti nues to end of the process, the lactic acid culture takes part predominantly during the second ripening period.

3.The intensity of the exogenic respiration of the starter cultures depend on their age and physiological condition while its maximum coincides with their active exponential phase of growth, which for the microccocus is developed on the 12th hour, and for the lactic acid culture on the 16th hour after innocula tion.

4.The endogenic respiration for both cultures show neglige able activity and is in relation

with the age of the culture, while for the micrococcus it is within the limits of 0,8 to 3,6 $mm^3/mg/hr\ O_2$, and for the lactic acid culture from 0,6 to 1,4 $mm^3/mg/hr\ O_2$.

5. Protein content for both cultures does not change in the lag phase and gradually increases in the active exponential phase (for the micrococcus to the 12th hour, and for the lactic acid culture to the 16th hour), after that an intensive protein biosynthesis is being observed in both strains, reaching its maximum in the stationary phase.

6. The best growth of the biomass for the starter cultures is determined in the active exponential phase, for the micrococcus to the 12th hour, and for the lactic acid culture to the 16th hour, which time coincides with the most intensive exogenic respiration and the transition to a sharp increase of the protein in the cells.

7. The established correlation between the growth dynamics, respiratory activity, and protein synthesis for the investigated two starter cultures characterises their bioenergetic and biosynthetic activity, proving in this manner their adaptility for use as starter cultures in the meat processing, acting in the processe of the ripening of the raw dried meat products.

8. Gulose and lactic acids in quantity as found in the meat products are used as substrates from both investigated strains, proving their help in the development in the ripening of the raw dried meat products.

9. The oxydation of the ascorbic acid in quantities as used in the meat processing, in the exogenic respiration of both strater cultures is negligeable, which in turns guarantees its part for the colour stabilisation of the meat products.

10. On the model solutions which contain aside from the substrates and substances having an inhibitor action, the oxydative

property of both strains is close th that of the lactic acid and glucose, which is explained with the presence of inhibitor substan ces in them.

11.A linear correlation is established bteween the quantity of used oxygen and the time for oxydation of all investigated substartes and model solutions from both cultures.

12.NaCl in concentrations as used in meat processing, dep resses the respiratory activity of the two investigated strains. With the rise of the concentration of the NaCl, the quantity of the used oxygen decreases inspite of the use of substrates.

13.For different substrates, NaCl depresses the oxydative property of both cultures to a different degree and this repre ssive effect is mostly exibited with the ascorb ic acid and in a weaker form with glucose and lactic acid.

14.The sensitivity of both investigated strains to the inhibitor action of NaCl, depends on thr age of the culture and is higher for the young in their active phase of division,cells in the initial exponential phase and decreases in the stationary phase.

15.$NaNO_3$, and$NaNO_2$ in concentrations as used in meat proce ssing, have a small stimulating effect on the exogenic respiration of the investigated cultures, which is stronger with a solution of 0,125% for $NaNO_2$ during the active exponential phase of growth.

16.$Na_4P_2O_7 \cdot 10H_2O$ in the investigated concentrations as used in meat processing,exibits only an inhibitor action on the respirator activity of both strater cultures under imvestigation, which is increased with the increase of the concentration.

Table 3

Substances in %	Exponential phase				Stationary phase			
	Quantity used O_2 $mm^3/mg/hr$		Stimulating respiration %		Quantity used O_2 $mm^3/mg/hr$		Stimulating respiration %	
	STRAIN 199/10	STRAIN 4669/6	STRAIN 199/10	STRAIN 4669/6	STRAIN 199/10	STRAIN 4669/6	STRAIN 199/10	STRAIN 4669/6
$NaNO_3$								
Exper. 0,025	47.42	41.96	0.00	0.78	24.00	13.50	0.00	0.00
0,08	48.01	42.86	1.24	2.96	24.00	13.68	0.00	1.32
0,25	48.41	42.98	2.08	3.24	24.25	13.77	1.04	1.98
$NaNO_2$								
Exper. 0.0125	48.71	43.27	2.72	3.98	24.58	13.69	2.42	1.38
0.125	49.35	44.44	4.06	6.74	24.75	13.89	3.12	2.92
$Na_4P_2O_7$	Quantity used O_2 $mm^3/mg/hr$		Depression of respiration in %		Quantity used O_2 $mm^3/mg/hr$		Depression of respiration in %	
Exper. 0,2	46.08	39.80	2.82	4.39	23.82	13.34	0.76	1.23
0.3	43.21	36.39	8.88	10.18	23.12	12.95	3.68	4.08
0.4	42.31	36.35	10.78	12.69	22.98	12.78	4.26	5.31
Control	47.42	41.63	0.00	0.00	24.00	13.50	0.00	0.00

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