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MICROBIOLOGICAL TRANSFORMATION OF HERBICIDES IN CATABOLIC PROCESSES

PART I. THE EFFECT OF VENZAR ON THE RESPIRATION ACTIVITY OF SELECTED GENERA OF SOIL BACTERIA [1]

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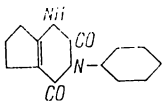

Venzar (3-cyclohexyl-5, 6-trimethyleneuracil) belongs to herbicides slightly soluble in water (at 25° 6 ppm). To weeds it is a photosynthesis inhibitor. Soil bacteria are little susceptible to this herbicide.

In the presence of *Bacillus* sp. venzar markedly increased the respiration and decreased the photosynthesis of leaves of *Sinapis alba* and *Spirodella polyrrhiza* as compared with the action of venzar itself and control [8]. Our aim was to test whether and to what extent venzar can influence the metabolism of bacteria resistant to high doses of this herbicide, and particularly their respiration activity.

MATERIAL AND METHODS

The tested bacterial strains were isolated from previously percolated soils : sandy-loamy soil, the Wrocław black earth, sandy soil Yugoslavian black-earth treated with low doses of venzar (1.5 ppm). The origin of individual bacterial strains, their genus affiliation as well as the conditions of isolation are given in Table 1. To isolate the strains of bacteria under examination the following media were chosen : the Kaufman synthetis medium [12], the Kearney synthetic medium [11], the Čapek medium [7] for *Pseudomonas,* Sands and Rovira [15] and a soil extract (flood with 1.5 l of tap water 1 kg of soil, sterilize after

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Table 1

List and origin of bacterial strains tested

Bacterial strain		Percolated soil + venzar	ppm	Culture medium for isolation + venzar	ppm
Symbol	Genus				
V-217	Bacillus sp.	loamy-sandy	0	Kearney	1000
V-218	Bacillus sp.	loamy-sandy+2% cellulose	0	Kearney	0
V- 92	Bacillus sp.	black degraded	1.5	Kearney	100
V- 25	Bacillus sp.	loamy-sandy	0	soil extract	100
V- 76	Bacillus sp.	loamy-sandy fertilized	3	Capek	100
V- 20	Bacillus sp.	loamy-sandy	9	synthetic, Kaufman	100
V- 16	Bacillus mycoides	loamy-sandy fertilized	3	Kaufman synthetic	100
V- 80	Pseudomonas fluorescens	Yougoslav black-earth	1.5	for Pseudomonas	1000
V-209	Pseudomonas fluorescens	Yougoslav black-earth+2% cellulose	1.5	for Pseudomonas	100
V-208	Pseudomonas fluorescens	Yougoslav black-earth+2% cellulose	1.5	for Pseudomonas	1000
V-105	Pseudomonas fluorescens	black degraded	1.5	Kearney	100
V- 65	Pseudomonas fluorescens	loamy-sandy fertilized	9.0	for Pseudomonas	100
V-212	Pseudomonas aeruginosa	Yougoslav black-earth+2% cellulose	0.0	for Pseudomonas	1000
V-204	Pseudomonas aeruginosa	loamy-sandy	1.5	for Pseudomonas	100
V-211	Pseudomonas aeruginosa	sandy	1.5	for Pseudomonas	1000
V-100	Pseudomonas aeruginosa	black degoaded	1.5	for Pseudomonas	1000
VI-5	Pseudomonas aeruginosa	loamy-sandy	0.0	for Pseudomonas	100
V-206	Pseudomonas aeruginosa	Yougoslav black-earth	1.5	for Pseudomonas	1000
V-223	Pseudomonas aeruginosa	loamy-sandy	1.5	for Pseudomonas	1000
V-23a	Pseudomonas aeruginosa	loamy-sandy fertilized	1.5	Kearney	1000
V-205	Pseudomonas aeruginosa	loamy-sandy	1.5	for Pseudomonas	0
V-11	Pseudomonas alcaligenes	loamy-sandy	9.0	Kaufman synthetic	1000
V-1	Pseudomonas alcaligenes	loamy-sandy	9.0	Kaufman synthetic	0
VIV-3	Pseudomonas species	black degraded fertilized	0.0	Kearney	1000
V-4a	Corynebacterium sp.	loamy-sandy	9.0	Kaufman synthetic	1000
V-84	Corynebacterium sp.	sandy	1.5	Kearney	100
V-36	Corynebacterium sp.	loamy-sandy	0	soil extract	100
V-216	Arthrobacter sp.	loamy-sandy	0	Kearney	1000
VVI-8	Arthrobacter sp.	black degraded fertilized	0	Kearney	100
V-5	Micrococcus sp.	loamy-sandy	9.0	Kaufman synthetic	100
VIX-1	Serratia sp.	black degraded fertilized	0	Kearney	1000
V-10	Nocardia sp.	loamy-sandy fertilized	0	for Pseudomonas	100

24 hours and filter the liquid through filter paper and enrich with KH_2PO_4 — 0.5 g/l, glucose — 10 g and agar 16 g, and then sterilize again). A part of it was enriched with 100 and 1000 ppm of venzar. A corresponding herbicide dose was suspended in a small volume ethanol and added to previously sterilized media. 32 bacterial strain coming from four successive microbiological analyses were examined, that is: 17 strains of genus *Pseudomonas*, 7 strains of genus *Bacillus*, 5 strains of genus *Corynebacterium*, one *Nocardia*, sp., one *Serratia* sp. and one Micrococcus sp.

The respiration measurements were made using the respirometric

method in the Warburg apparatus at 27°C in vessels of 3 ml volume [5, 6]. One-day liquid broth cultures of individual bacterial strains were centrifuged (10 000 r.p.), and washed with psysiological salt solution, as well as suspended in a phosphate buffer of pH 7.2. One millilitre of this suspension was introduced into the main chamber of the vessel. The same amount of suspension was dried at 105°C up to a constant weight in order to determine the dry mass of cells which was between 10 and 12 mg/ml. Into the side arms of the Warburg vessels the respiration substrates were introduced : a solution of watersoluble dose of venzar (6 ppm) and 0.1% glucose solution, or mixtures of both these compounds. For each bacterial strain the oxygen uptake and carbon dioxide evolution in the presence of the above-mentioned respiration substrates were examined, and the respiration factors RQ were calculated. The measurements were usually carried out over 8 hours, reading out the level of manometers every half an hour [17]. Apart from the exogenic respiration, endogenic respiration was also examined. The results of the gas exchange were expressed in microlitres (µl) of the taken up or evolved gas for one mg of dry mass of bacteria. On the basis of the obtained results the oxygen uptake curves and the carbon dioxide evolution curves were plotted.

By the method of thin- layer chromatography the undegraded herbicide and its metabolites in the liquid from the Warburg vessels was determined after completion of gas exchange investigation. Venzar was extracted with ethyl acetate, and the chromatographic plates covered with silica gel G-60 were developed in the system benzene : acetone (6 : 1) and then sprayed with a starch reagent [14].

RESULTS AND DISCUSSION

The effect of venzar on the respiration activity of various genera of bacteria was different (Figs 1–7). Most bacteria of genera *Bacillus* (Fig. 2), *Pseudomonas* and *Arthrobacter* (Figs 1, 4) show ability to oxidation of venzar to equal degree as glucose and in some cases venzar was even better utilized (Figs 1, 2). The bacteries of genus *Pseudomonas aeruginosa* and one strain *Arthrobacter* sp. very intensely oxidated the mixtures of glucose and venzar (Fig. 4).

The respiration factors RQ for bacteria oxidating venzar were higher than this would result from the stechiometric reaction (Table 2)

$$C_{13}H_{18}O_2N_2 + 16.5\ O_2 \rightarrow 13\ CO_2 + 9H_2O + N_2$$

$$RQ = \frac{13\ CO_2}{16.5\ O_2} = 0.78$$

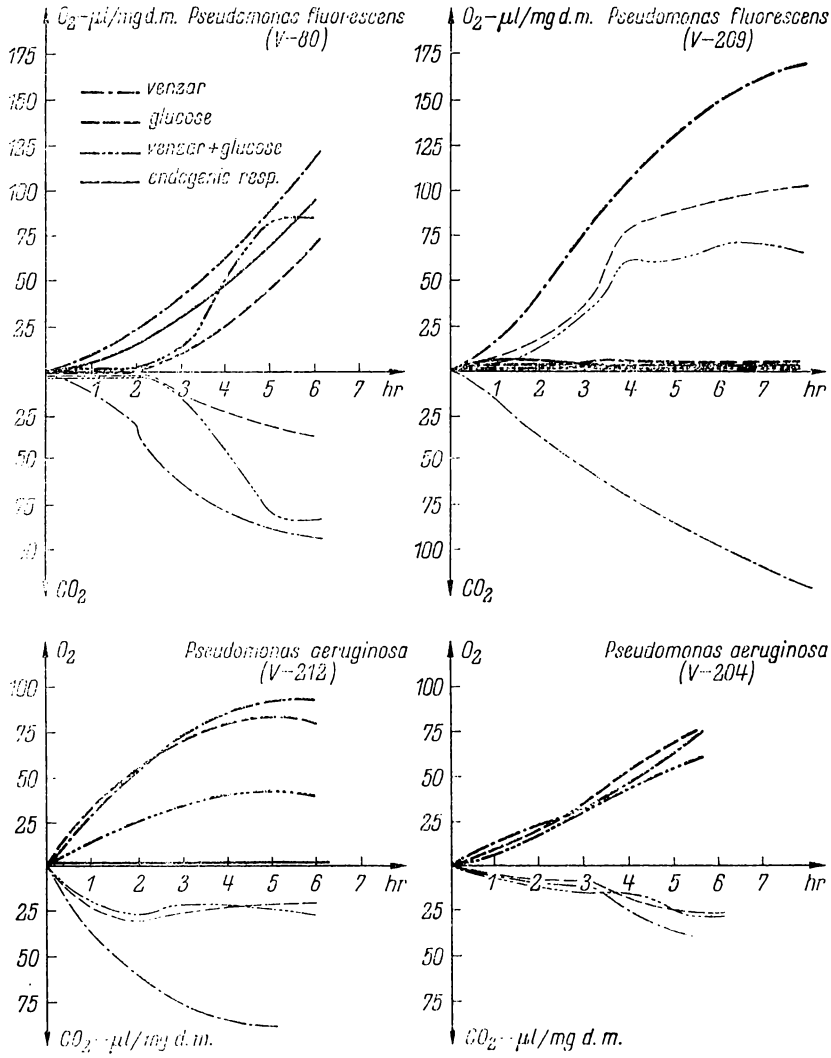

Fig. 1. The respiration of *Pseudomosa* strains utilizing various carbon sources

The genus *Pseudomonas* prevailed quantitatively in the population of isolated bacteria resistant to high doses of venzar in the substrate (100 and 1000 ppm) and most intensely utilized this herbicide as a carbon source (Fig. 1). After completion of the investigation of gas exchange in the liquid from the Warburg vessels, in most cases no residues of undegraded herbicide were detected, while there appeared new metabolites (Table 2). For the genus *Bacillus* venzar was also a carbon source. A decrement of about 75% of this herbicide was noted chromatographically but no metabolites were detected. Factors RQ were higher for venzar, which could indicate the predominance of decarbo-

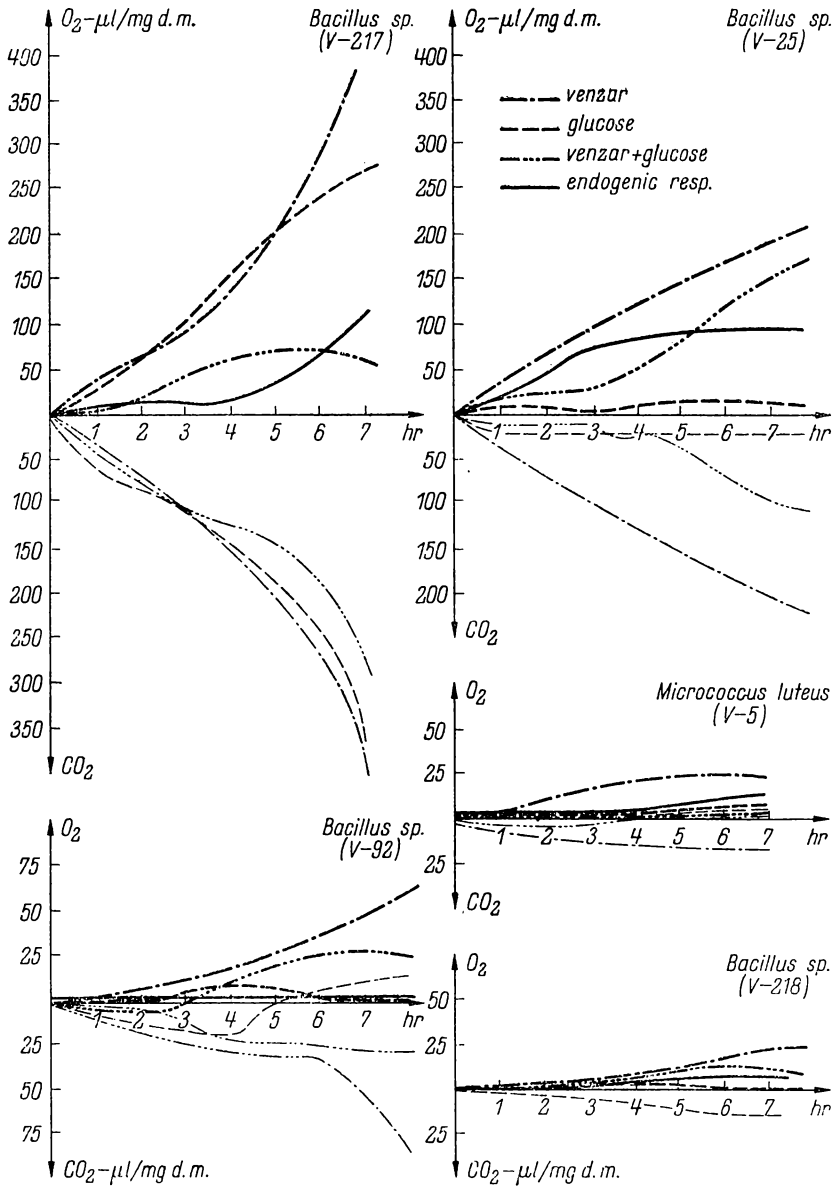

Fig. 2. The respiration of *Bacillus* sp. and *Micrococcus* sp. strains — various carbon sources

xylation processes, incomplete oxidation of the herbicide and of the evolution of yet other gases besides CO_2, or of oxidation typical of organic acids which could be formed in the degradation process of venzar. It is not known which of the possibilites mentioned was the cause of increased values of the respiration factors RQ.

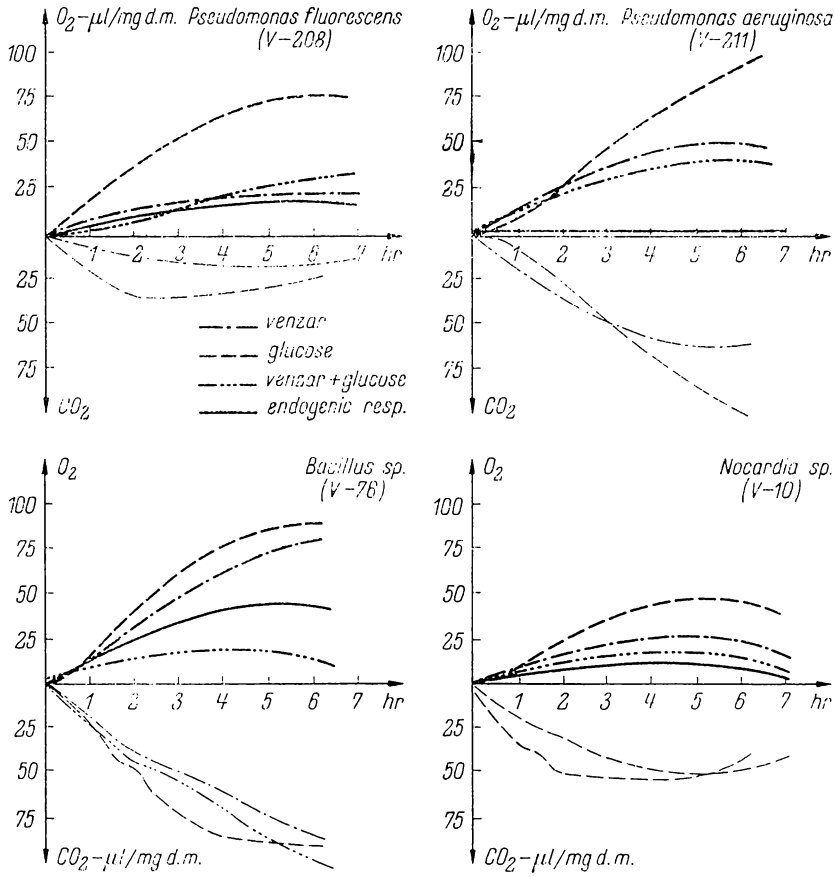

Fig. 3. The effect of glucose and venzar on the respiration of various bacterial strains

The other genera of bacteria : *Corynebacterium, Serratia, Nocardia, Micrococcus, Arthrobacter* and *Pseudomonas* alcaligenes oxidated venzar more poorly (Figs. 2, 5, 6, 7) or they could not adapt themselves to the utilization of venzar as a respiration substrate. Thus they have shown a very small respiration activity. At the same time a disturbance in respiration processes was observed, manifesting itself by breakdown and stoppage of respiration, the respiration factors RQ reaching in these experiments irrational values (Table 2). The above-mentioned results indicate that venzar changed the metabolism of these bacterial strains

A water-soluble dose of venzar (6 ppm) acted stimulating on the respiration of some of bacteria of genera *Pseudomonas, Bacillus* and *Arthrobacter* (Figs. 1, 2, 4), the stimulation being proportional to both the oxygen uptake and CO_2 evolution. Other herbicides, as reported

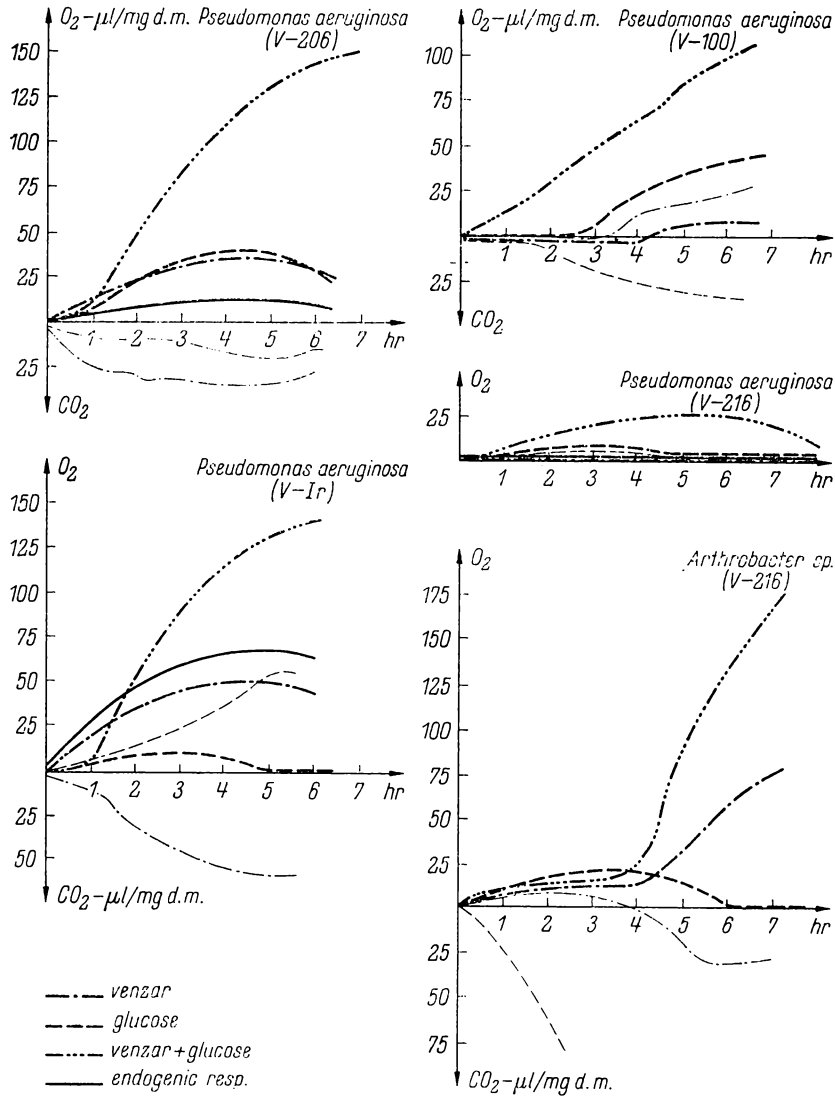

Fig. 4. The effect of glucose and venzar mixture on the respiration on *Pseudomonas aeruginosa* and *Arthrobacter* sp. strains

by Karpiak, Iwanowski [10], can stimulate or inhibite disproportionately oxygen and oxygen-free processes.

Generally, one can say that 50% of the bacteria examined were susceptible to the presence of venzar, which manifested itself by a disturbance in respiration processes. In this group were present forms of *Corynebacterium* sp., *Serratia* sp., eight strains of genus *Pseudomonas* sp. and two strains of genus *Bacillus* sp. The other bacterial strains of genus: *Bacillus*, *Pseudomonas* and *Arthrobacter* utilized venzar as

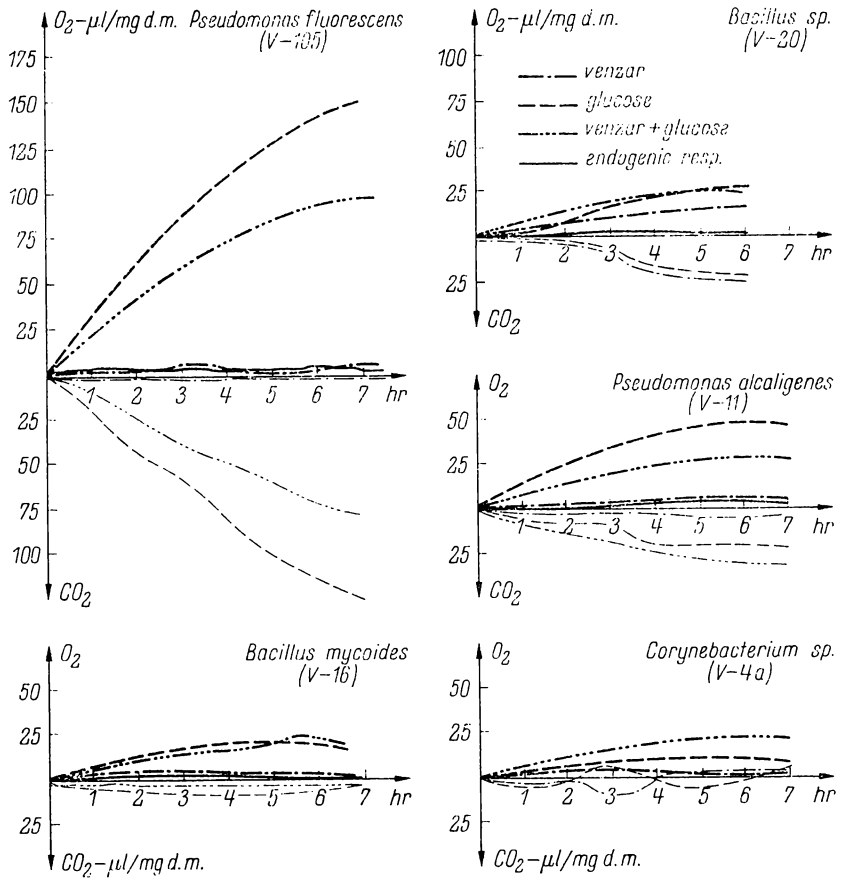

Fig. 5. The effect of glucose and a mixture of glucose and venzar on the respiration of various bacterial strains

a substrate in processes of oxygen respiration, thus contributing to its removal from the medium.

The obtained results stress the necessity of further investigations on microbial degradation of herbicides, which we will carry out in our successive experiments whose justification is also found in the studies by: Alexander [1, 2], Miayamoto and Ontavoi [13], Ballag [3, 4], Gołąb, Althans [9], Sobieszczański [16].

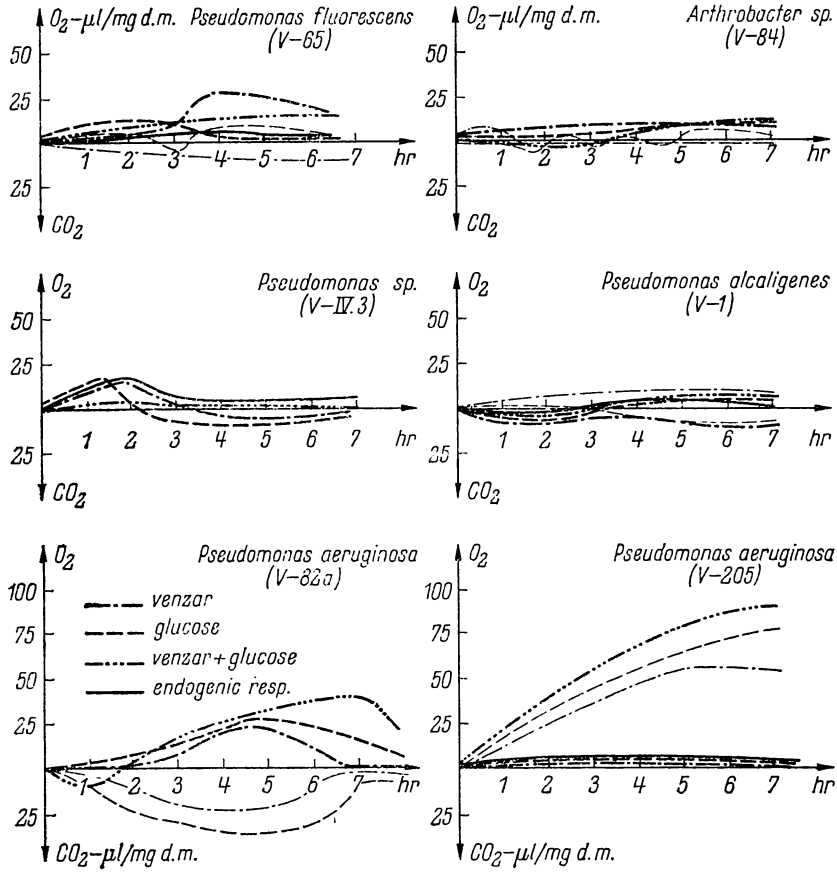

Fig. 6. The disturbance in respiration of *Pseudomonas* and *Arthrobacter* sp.

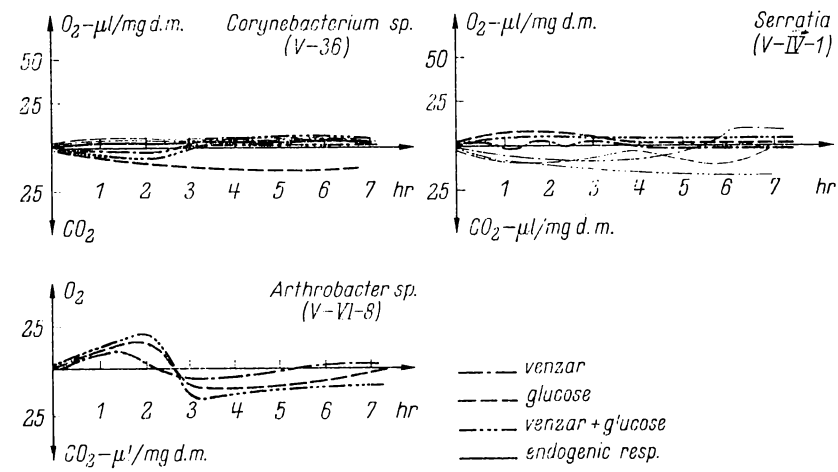

Fig. 7. The disturbance in respiration of *Corynebacterium* sp.

Table 2

The effect of venzar and glucose on gas exchange of the tested genera of bacteria and the decrement of venzar from the medium

Bacterial strains		RQ Respiration substrates			Venzar residue	
N°	Genus	venzar 6 ppm	glucose 0.1%	% venzar + + glucose	%	Rf of metabo - lites
V-217	Bacillus sp.	1.17	5.30	2.90	0	0.39 0.92
V-218	Bacillus sp.	0.91	1.40	1.09	70	———
V-92	Bacillus sp.	0.98	1.27	1.05	20	———
V-25	Bacillus sp.	1.12	1.27	0.42	25	———
V-76	Bacillus sp.	1.20	1.12	1.40	20	———
V-20	Bacillus sp.	1.27	0.81	-	0	———
V-16	Bacillus mycoides	1.06	0.29	0.20	98	———
V-80	Pseudomonas fluorescens	1.08	0.68	0.82	0	0.47 0.66 0.94
V-209	Pseudomonas fluorescens	0.70	irrational	-	0	0.25 0.40 ___
V-208	Pseudomonas fluorescens	0.64	0.47	2.10	20	0.29 ___ ___
V-105	Pseudomonas fluorescens	1.30	0.67	0.75	100	———
V-65	Pseudomonas fluorescens	0.68	0.84	-	95	———
V-212	Pseudomonas aeruginosa	1.00	0.35	0.70	0	0.47 0.88 0.95
V-204	Pseudomonas aeruginosa	0.44	0.33	0.38	0	———
V-211	Pseudomonas aeruginosa	1.30	1.00	-	0	0.17 0.37 ___
V-100	Pseudomonas aeruginosa	1.60	0.82	-	20	———
VI-5	Pseudomonas aeruginosa	1.12	7.40	-	50	0.32 ___ ___
V-206	Pseudomonas aeruginosa	0.91	0.52	-	50	0.32 ___ ___
V-223	Pseudomonas aeruginosa	1.20	2.50	-	100	———
V-82a	Pseudomonas aeruginosa	0.38	0.28	0.47	90	———
V-205	Pseudomonas aeruginosa	1.40	4.40	-	0	———
V-11	Pseudomonas alcaligenes	1.05	0.45	1.18	0	———
V-1	Pseudomonas alcaligenes	1.17	1.00	-	0	———
VIV-3	Pseudomonas species	-	-	-	0	———
V-4a	Corynebacterium sp.	11.80	0.25	-	25	———
V-84	Corynebacterium sp.	0.12	0.26	-	95	———
V-36	Corynebacterium sp.	0.19	0.25	7.30	100	———
V-216	Arthrobacter sp.	0.36	21.7	-	20	———
VVI-8	Arthrobacter sp.	-	-	-	0	———
V-5	Micrococcus	-	-	1.08	25	———
VIV-1	Serratia	4.4	1.6	4.30	50	———
V-10	Nocardia	2.07	1.12	-	20	———

CONCLUSIONS

1. Bacteria of genera : *Bacillus, Pseudomonas, Nocardia, Corynebacterium, Serratia, Micrococcus* and *Arthrobacter* are susceptible to high doses of venzar (100 nad 1000 ppm) in the medium.

2. Most bacteria of genera : *Psedomonas Bcillus* and *Arthrobacter* can utilize this herbicide as a carbon source in energetistic processes, thus contributing to the removal of venzar from the medium.

3. Venzar at 6 ppm stimulated respiration processes in bacteria of genera : *Bacillus, Pseudomonas, Arthrobacter.*

4. In some of genera of bacteria (*Corynebactrium* sp., *Serratia* sp., *Nocardia* sp., *Micrococcus* sp.) venzar disturbed respiration processes.

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J. SOBIESZCZAŃSKI, A. RODZIEWICZ, R. STEMPNIEWICZ

MIKROBIOLOGICZNA TRANSFORMACJA HERBICYDÓW W PROCESACH KATABOLICZNYCH

CZĘŚĆ I. WPŁYW VENZARU NA AKTYWNOŚĆ ODDECHOWĄ WYBRANYCH RODZAJÓW BAKTERII GLEBOWYCH

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we Wrocławiu

Streszczenie

Badano 32 szczepy bakterii wyodrębnione z różnych gleb uprzednio perkolowanych wodą zawierającą niską zawartość venzaru [tab. 1]. Szczepy te należały do rodzajów: *Pseudomonas, Bacillus, Corynebacterium, Serratia, Nocardia, Micrococcus* i były oporne na dawkę 100 i 1000 ppm venzaru w podłożu. Posługując się bezpośrednią metodą Warburga badano proces oddychania szczepów bakterii oznaczając ilość pobieranego tlenu i wydzielanego dwutlenku węgla w obecności venzaru, glukozy i mieszaniny obu substancji jako substratów oddechowych. Venzar okazał się dobrym substratem oddechowym dla większości bakterii z rodzajów: *Pseudomonas, Bacillus, Arthrobacter* i stymulował oddychanie tych szczepów bakterii. Pozostałe szczepy bakterii wykazały w obecności venzaru niską aktywność oddechową i zaburzenia procesów wymiany gazowej.

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