EFFECT OF KRILIUM ON THE RESPIRATORY ACTIVITIES OF *RHIZOBIUM TRIFOLII* AND *AGROBACTERIUM TUMEFACIENS* ON VARIOUS SUBSTRATES

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INTRODUCTION

Considerable attention has been given to the application of synthetic resins as a means of developing good soil structure. The use of such compounds in the soil has raised the question as to their possible effect on soil microorganisms. Productive capacity of a soil is greatly influenced by the texture and structure of that soil. The term texture is used in reference to the type of particles forming the soil, i.e. the proportion of clay, sand and silt, amount and type of organic matter present in the soil particles. The structure of the soil refers to the arrangement and position of the soil particles. Structure of the different horizons of a soil profile is an essential characteristic of a soil just as are the texture and chemical composition. Moisture relations, availability of plant nutrients, action of microorganisms and plant growth are all greatly influenced by the structure of the soil.

Formation of Soil Aggregates - There are individual or single particles found in the soil which have to be brought together to form the stable aggregates necessary for good

* Krilium is the trade name of a synthetic polyelectrolyte resin manufactured by Monsanto Chemical Company.

soil structure. In order for these particles to be formed into aggregates some means of cementing or holding these particles together must be provided. It is the mechanism of the formation of these aggregates which is one of the most important phases of the soil structure problem. It has been pointed out (11) that stable aggregate formation cannot take place in the soil unless some type of soil colloidal material is present, which indicates that it is the soil colloidal material which is responsible for the formation of primary particles into stable aggregates. Three groups of colloidal matter have been shown to be responsible for the formation of the aggregates (10). They are (1) clay particles, (2) irreversible or slowly reversible inorganic colloids, such as the oxides of iron and alumina, and (3) organic matter. Of the three groups, the clay particles and organic matter are of the greatest importance in the formation of soil aggregates.

One of the most characteristic products making up the soil organic matter is known as humus. Good stable aggregation has been found where there is a high percentage of humus present in the soil. Humus has been shown (11) to possess the ability to form soil aggregates which are necessary for good soil structure. It is a complex substance which is now known to be produced in at least three ways (10) (a) by bacterial attacks on cellulose, (b) by chemical transforma-

tions of lignin and (c) from fungal mycelia. Among the humus constituents are the polyuronides and uronic groups that are widely distributed in plants, composts and soils (10). It is a known fact (10) that certain gums of the polysaccharide or polyuronide types occurring in good topsoil or humus are responsible for cementing together primary soil particles to form water stable aggregates of optimum size.

Aggregation By Synthetic Compounds - Several chemical companies have recently initiated research projects in an effort to develop synthetic compounds which could be used to supplement or replace some of the organic matter of soils so as to develop better soil structure and, in general, more desirable chemical and physical properties of the soil. Monsanto Chemical Company happened to be the first to place a synthetic soil conditioner on the market, which they gave the trademark of Krilium. Krilium is a synthetic polyelectrolyte resin, which according to the manufacturer, can be used as a replacement for the natural polysaccharide or polyuronide resins derived from humus. It should be noted that the production of this compound has just recently been changed from a pilot plant to a large production basis.

The mechanism by which Krilium causes the formation of soil aggregates is principally the same as that of the natural organic colloids of the soil. Krilium exists as a polyanion with a hundred or more negative charges on the indivi-

dual "ions". The hygroscopic action of Krilium helps it to become solubilized in the soil water when it is dispersed in the soil. The polyanions presumably are absorbed to colloidal particles, binding these through the carbon linkage "bridge" between reactive groups on the polymer. To bind the ultimate particles of the soil together by means of the carbon linkage is the basis of the soil conditioning effect. Maximum aggregating effect of Krilium at lowest concentrations are obtained in the presence of small amounts of sodium, calcium, magnesium and other cations (6).

Krilium has only been publicized for approximately nine months. Monsanto Chemical Company has had cooperation with universities, federal and state agencies in directing studies toward evaluation of effects and determinations of the most efficient methods of use. Since Krilium is so new, the research laboratories have not had sufficient time to publish papers in regard to results obtained with Krilium. The manufacturer has published a bulletin (13) in which, from preliminary investigations, a discussion is given of the properties, possibilities and uses of Krilium. This bulletin is the only source of information to date pertaining to the actions of Krilium. It should be noted that from a survey of available literature no evidence could be found as to the effect of Krilium on the soil organisms.

In the above mentioned bulletin (13), it has been pointed out that one pound of Krilium appears to be equivalent to

the natural gums produced by 100 to 1000 pounds of manures or plant residues. It is also noted in this bulletin that Krilium has no undesirable effects on the bacterial population of the soil and that it retains its aggregating power against decomposition by soil microorganisms in some cases at least 10 times as long as the natural organic matter.

Effects of Krilium on Soil - Mention has been made (6) (13) of the effects Krilium has on the soil and some of these are itemized below:

(1) Soil aggregation is often doubled or tripled after treatment with Krilium.

(2) Soils treated with Krilium have increased ability to give higher yields and improved quality of crops.

(3) The moisture equivalent of the soil is increased up to 50% so as to make more water available to the plants growing on the treated soil.

(4) Moisture loss due to evaporation is retarded.

(5) Soil aeration is significantly improved resulting in a better supply of oxygen to the plant roots.

The manufacturer reports that Krilium will be effective when used in concentrations ranging from .02% to .10% by weight of the soils treated. They recommend a concentration of .05% by weight of soil as a good working concentration. It is suggested (13) that concentrations above .2% may be detrimental.

Purpose of Investigation

The primary purpose of this investigation was to study the effect that various concentrations of Krilium might have on the respiratory activities of the following two soil organisms:

(1) *Rhizobium trifolii*

(2) *Agrobacterium tumefaciens*.

A secondary objective was to observe any possible differences of the effect of varying concentrations of Krilium (Blend #6) and Krilium (Blend #9) on the respiratory activities of the above mentioned organisms.

These organisms were chosen to be used in this study because both are concerned in agricultural practices - the first one as root nodule bacteria on clover, and the second as a plant pathogen that causes crown gall.

METHODS

Manometric methods have proven to be one of the most useful means for studying cellular activities. There are several modifications of manometric instruments available for determinations of the evolution or absorption of gases by cells. One of the most extensively used modifications is the Warburg respirometer, which was employed in this investigation (3)(14).

The "resting cell" technique (15) was used in making this study of the respiratory activities of the test organisms. This technique involves the use of non-proliferating cells (washed suspension of cells) which can be used to obtain results of respiratory rates without the influence of growth on the respiration of the organisms. Many previous studies (4)(9) of the respiratory activities of bacteria have been made with growing cultures, but since there is no means of interpreting the effects of growth on the respiration rates, the difficulty arises as to how to interpret the data obtained. The "resting cell" technique is, therefore, the more generally used method of studying respiratory activities of bacteria.

Warburg Respirometer

Theory of Warburg Respirometer - The Warburg apparatus is one of the many types of manometric instruments which

have been used in estimating the exchange of gases of biological and chemical reactions. This type of respirometer has possibly been used more extensively than any other type of respirometer in biological studies. The main principle involved is that at constant temperature and constant gas volume any changes in the amount of gas in the flask can be measured by changes in its pressure.

The Warburg apparatus consists essentially of a U-tube manometer and a reaction flask. The manometer has one end open to the atmosphere and the other end attached to the reaction flask. Attached to the manometer is a tygon tube which serves as a reservoir for the manometric liquid of known density. There is also provided a screw clamp which is used to adjust the level of the liquid in the U-tube. The reaction flask has one or more sidearms and a center cup. More complete details of the apparatus may be found in the literature (3)(14).

When the respirometer is being used, the reaction flask with its contents is attached to the manometer. The apparatus is so designed that the manometer can be attached to a shaking apparatus and at the same time allow the reaction flask to be completely immersed in a water bath. The manometer is on the outside of the water bath and is so positioned to make it easy to observe any change in the level of the manometer fluid. The flasks are immersed in the

water bath at a constant temperature and shaken to promote a rapid exchange of gas between the liquid and gas phases.

Before the Warburg apparatus can be used in estimating changes in the amount of any gas in the flask the volume of the manometer and flask must be determined. So that a constant volume can be established and maintained, the level of the liquid in that side of the manometer which is attached to the flask is always adjusted to the same point (in this case to 150 mm.).

A known amount of liquid in which the reaction will take place is contained in the flask. This reaction may either involve an evolution or absorption of a gas. The change in volume of the gas can be determined by observing the level of the manometer fluid both before and after the reaction has taken place. This difference in the level of the manometer fluid is due to the change of the pressure inside of the reaction flask.

The actual change in the amount of gas can be calculated by the equation: $x = hk$

where, x is the amount of gas evolved or absorbed at normal pressure and temperature. If the gas is evolved, x will be positive and if the gas is absorbed, x will be negative.

h is the reading of the open arm of the manometer in millimeters.

k is the apparatus constant, usually known as the flask constant. This constant must be known in order to convert mm. pressure change into microliters of gas involved in the reaction. This constant, k, varies with the conditions of the experiment and for a given constant these conditions must remain the same. Also k may or may not be the same for each individual flask (3)(14).

There are several methods which could be used to calibrate the apparatus, which in turn is the determination of the "flask constants" (3)(14). Three methods for calibration of the apparatus are as follows:

1. By the Kunzer and Newman method (3) in which a measured amount of gas is added or withdrawn from the flasks by means of a graduated pipette, and the resulting reading of the manometer observed.
2. By liberating or absorbing a known amount of gas in the vessel by means of a chemical reaction.
3. By calculation from the following formula:

$$k = \frac{V_g \frac{273}{T} + V_f a}{P_o}$$

where,

V_g = Gas volume

V_f = Liquid volume in the flask

T = Temperature of water bath in absolute degrees. (273 temperature in $^oC.$)

a = Solubility in liquid in vessel of gas involved (expressed as ml. gas/ml. liquid when gas is at a pressure of one atmosphere (760 mm. Hg) at the temperature T.

P_o = 760 mm. Hg (standard pressure) expressed in terms of the manometer fluid:

P_o = 760 X 13.6 (specific gravity of Hg)/specific gravity of manometer fluid.

The method used to determine the constants in this work was by liberating a known volume of gas and making use of the preceding formula in order to determine the constants.

Determination of Oxygen Absorption - There are two methods of measuring the oxygen uptake of cells by manometric methods: (1) the method by which the oxygen uptake is measured directly and (2) the method by which it is obtained by indirect calculation. The former method was used in this

study. In the direct method the center well of the flask contains alkali which absorbs the carbon dioxide produced by the respiring cells, so that the carbon dioxide liberated has no effect on the manometer. The reading of the manometer then gives a direct measure of the oxygen obsorption. Using the direct method, only one flask is necessary to determine oxygen absorption.

The concentration of the alkali used by various investigators varies but KOH is almost universally employed as the alkali because of the solubility of potassium carbonate. A 15% solution of KOH was used to insure sufficient concentration of KOH to obtain instantaneous absorption of carbon dioxide. To increase the surface area of the alkali, a square of filter paper with 2 cm. sides was folded in accordion fashion and placed in the center cup.

Determination of Carbon Dioxide Liberation - The direct method for determining carbon dioxide liberation was used in this investigation. This determination necessitates the use of two flasks respiring in the same way, except that in one, the carbon dioxide is absorbed whereas in the other it was not. The flask containing the alkali provides a means of measuring the oxygen uptake which needs to be known in order to calculate from the second flask the amount of carbon dioxide liberated. Carbon dioxide liberation can be calculated as follows:

Let X_{O_2} = amount of oxygen absorption

X_{CO_2} = amount of carbon dioxide liberated.

The manometer change due to oxygen absorption is

$$h_{O_2} = X_{O_2}/k_{O_2} \text{ since } X_{O_2} = h_{O_2} k_{O_2} .$$

The manometer change due to carbon dioxide production is

$$h_{CO_2} = X_{CO_2}/k_{CO_2} .$$

So the final observed reading h in the flask without KOH would be the resultant of the two,

$$h = h_{O_2} + h_{CO_2} = X_{O_2} / k_{O_2} + X_{CO_2} / k_{CO_2}$$

thus,

$$X_{CO_2} = (h - X_{O_2} / k_{O_2}) k_{CO_2}$$

X_{O_2} is known from the flask which contained KOH and k_{O_2} and k_{CO_2} are known from the flask without KOH, h being observed, hence X_{CO_2} can be calculated.

Thermobarometer - Since one end of the manometer tube is open to the atmosphere the Warburg manometers are very sensitive to slight changes in the barometric pressure or the temperature of the water bath. It is then necessary to have an additional manometer to serve as a thermobarometer. It is not necessary to calibrate the flask used as the thermobarometer but any change of the manometer level of the thermo-

barometer must be used in correcting the other manometers. If the level of the liquid in the open arm of the thermobarometer rises there has either been an increase in the temperature of the water bath or a decrease in the barometric pressure. If so, the number of mm. change must be added to the manometer readings of the other flasks in order to carry out an accurate determination. If there is a decrease in the liquid level of the open arm of the thermobarometer then the amount of change in mm. must be subtracted from the manometer readings of the other flasks.

Calibration of The Warburg Flasks - The method used to calibrate the Warburg flasks and manometers was that of releasing a given amount of a gas (CO_2) in the reaction flasks and by means of known equations (14) was able to calculate the constants and volumes of the manometers and flasks. The carbon dioxide was released by the addition of hydrochloric acid to sodium bicarbonate as shown by the following equation:

$$NaHCO_3 + HCl = NaCl + H_2O + CO_2\uparrow$$

A given amount of sodium bicarbonate was dried in an oven at 90°C. until a constant weight was obtained. After drying the sodium bicarbonate to a constant weight, three aliquotes were taken from the initial amount and were used in the determinations. The aliquotes were weighed on an analytical

balance and added to distilled water as follows:

(1) 0.7188 grams/liter

(2) 0.7188 grams/liter

(3) 0.5258 grams/liter

The calculation of the theoretical amount of gas to be liberated in each case was determined by the following equation:

$$X_{CO_2} = \frac{\text{wt. of } NaHCO_3 \times 22.4 \times 10^6}{\text{molecular wt. of } NaHCO_3}$$

Procedure of Calibration - The procedure followed to calibrate the respirometer was to place 1 ml. of 1N HCl in the sidearm of the reaction flask and 1 ml. of the $NaHCO_3$ solution in the main chamber of the flask. After allowing the flask to equilibrate for fifteen minutes, the flask was tilted so as to permit entrance of the HCl into the main portion of the flask. This initiated the reaction of the acid on the sodium bicarbonate which would release the carbon dioxide necessary for the determination. By observing the change in the level of the manometer fluid it was possible to use this figure to determine the flask volume and constants of the flask. In this case the flask volume refers to the volume of the flask plus that volume of the manometer from the level of the manometer liquid in the closed side. The heighth of the liquid was 150 mm. in each determination.

The constants of the flasks could be calculated since the theoretical amount of gas liberated (x) was known and h was observed. The constant (k) could be determined by $k = x/h$. Five determinations were made on each flask and the gas volume of each flask was determined as the average of these determinations. The flask volume could then be calculated by $V_g + V_f$.

Throughout the entire experimental work, the flasks and manometers were paired as shown below with the constants for each at specified conditions.

Table I

Values For Respirometer Volumes and Constants

Manometer	2	3	4	5	6	7
Flask	10	11	12	13	14	15
Volume (ml.)	17.3190	17.929	17.548	17.401	17.328	17.592
K_e 34°	1.5754	1.6308	1.5962	1.5829	1.5762	1.6185
K_{CO_2} (2 ml, 34°)	1.5146	1.5725	1.5430	1.5245	1.5179	1.5411
K_{O_2} (2 ml, 34°)	1.3984	1.4540	1.4245	1.4060	1.3993	1.4233
K_{CO_2} (3 ml, 34°)	1.4879	1.5419	1.5087	1.4953	1.4887	1.5115
K_{O_2} (2 ml, 34°)	1.3101	1.3641	1.3309	1.3175	1.3109	1.3348

The calculations were made using the following equation:

$$k = \frac{V_g \frac{273}{T} + V_f a}{P_o}$$

The symbols are the same as were shown on page 9 and page 10. The method of calibration was taken from Umbreit (14) and Dixon (3).

In order to calculate P_o it was necessary to determine the density of the manometer fluid. This was done by means

of a pycnometer. P_o was then calculated as shown below:

$$P = 760 \times 13.6/1.0574 = 9775$$

Products And Handling

Organisms - The two organisms used in this research were Rhizobium trifolii 205 (W. B. Sarles, American Type Culture Collection) and Agrobacter tumefaciens (Hendrickson, Strain A-6). These cultures were carried in stock on yeast extract mannitol mineral salts agar. In preparation of the cellular suspensions to be used for the Warburg studies, the bacteria were grown on yeast extract mineral salts agar which contained no carbohydrate source. By using this medium, it was possible to deminish the production of a polysaccharide gum which is characteristic of the above organisms when grown on a medium containing an available carbohydrate. Some of the reasons for attempting to inhibit the formation of this gum are given as follows:

1. The gum is not readily removed from the cells.
2. Makes the recovery of the organisms from the agar rather difficult.
3. The endogenous respiration (respiration of organisms without a substrate) is not too excessive.
4. Preparations used where there has been excessive gum formation are almost useless for the study of oxygen uptake, since most of the respiration arises from cellular constituents rather than from the substrate.

Substrates - The study of the effect of Krilium on the respiratory rates of the two soil organisms was made on three substrates to which Krilium had been added. A .02M solution of glucose, succinate and mannitol were used in the investigation. The given amount of each substrate was dissolved in a standard buffer solution (5).

Two blends of Krilium were used in the study, Blend #6 and Blend #9. Respiratory activities of the two organisms were first observed on glucose, succinate and mannitol in order to measure the rates of activities without the influence of Krilium. The respiratory activity was then observed when the organisms were subjected to the same substrates in the presence of different concentrations of the Krilium - .05%, 0.10% and 1.00%. Measurements were also made of the activity of the test organisms on the above concentrations of Krilium without another substrate.

Cell Suspension - In order to obtain valid results the cell suspension used in each run had to be standardized as nearly as possible, both in quantity of cells and the activity of the cells.

For each run a fresh suspension of cells was prepared so that the cells used each time would have approximately the same rate of respiration on the same substrate. The organism Rhizobium trifolii was permitted to grow for 44-48 hours before being used in a run. The culture Agrobacterium tume-

faciens was used in the determinations after a growth period of 18-24 hours. Rhizobium trifolii was permitted a longer period of incubation than Agrobacterium tumefaciens because the former organism has a much slower growing rate than the latter organism. Both organisms were harvested from the medium by being washed off with standard buffer solution. The culture suspension was then centrifuged with an International Clinical centrifuge with a speed of 2000 - 3000 revolutions per minute for a period of fifteen minutes. The cells obtained after centrifugation were resuspended in buffer solution and recentrifuged. This washing procedure was performed three successive times for each cell suspension to be used in one study. After the final washing the cells were resuspended in buffer solution to the desired concentration of cells (1.5 mg. cells/ml. by dry weight) to be used for the respiratory studies. The cellular suspension was adjusted to the proper concentration by turbidity measurements in a Klett-Summerson photoelectric colorimeter.

Calibration of the Klett-Summerson colorimeter was made in terms of dry weight versus turbidity reading. The dry weight versus turbidity curve for each organism was determined in the following manner:

(a) Each organism was grown in large quantities so as to prepare a very turbid suspension of cells.

(b) Four aliquotes of 5 ml. each was obtained from the

very turbid suspension.

(c) Three of the above aliquotes were put into tared 30 ml. beakers and were placed in an oven to dry at 90°C for a period of 36 - 48 hours. They were then placed in a desiccator and allowed to come to a constant weight. The dry weight of each aliquote of cells was determined.

(d) A turbidity reading was made of the fourth aliquote on the Klett-Summerson colorimeter. Turbidity readings were then made on the suspension after increased dilutions were made to the 5 ml. suspension.

(e) The weights of the three dried aliquotes were averaged to obtain the approximate dry weight of the cells in the suspension used to procure the turbidity readings.

(f) Making use of the determined dry weight of the cells in suspension and after making the turbidity readings, it was possible to plot the dry weight versus turbidity curve for each organism.

The standard suspension used in this work was 1.5 milligrams of cells per milliliter of buffer solution. The curves plotted for each organism used in this research are shown in Figures 1 and 2.

Warburg Determinations and Conditions - The Warburg re-

Figure 1. Turbidity - Dry Weight Curve for Rhizobium trifolii.

Figure 2. Turbidity - Dry Weight Curve for Agrobacterium tumefaciens.

spirometer was used to measure the oxygen consumption and carbon dioxide liberation of the test organisms on the given substrates. The temperature of the water bath was 34°C and the flasks were shaken 110 complete one inch strokes per minute. All determinations were made in an atmosphere of air. In all cases the fluid volume of the flasks was 3 m. (1 ml. of cell suspension, 1 ml. buffer and 1 ml. substrate). In the flasks used for the determination of oxygen uptake, .2 ml. of 15% KOH was placed in the center cup of the reaction flask to absorb the carbon dioxide liberated during the reaction.

Replications were made of each determination of the respiratory activities of the organisms on the various substrates in presence of concentrations of Krilium. A fresh suspension of cells was prepared for each replication. Only one determination was made on a specific substrate (i.e. glucose with .10% Krilium) with a portion of a given cell suspension. Endogenous oxygen uptake was determined for each run.

The temperature of the water bath was maintained at 34°C because the rate of respiration of the two organisms used is near optimum at this temperature and this temperature is sufficiently low so that inactivation of enzymes through prolonged exposure to higher temperatures is prevented (15).

Buffer Solution - The buffer solution used in this investigation was a KH_2PO_4 and NaOH buffer with a pH of 6.8 (5).

EXPERIMENTAL

Respiratory rates were determined for Rhizobium trifolii and Agrobacterium tumefaciens on specific substrates (glucose, succinate and mannitol) in the presence of various concentrations of Krilium. Two blends of Krilium were used in this experimental work, blend #6 and blend #9.

Values for the oxygen consumption and carbon dioxide liberation of Rhizobium trifolii respiring on the various substrates and in the presence of varying concentrations of Krilium, blend #6 and blend #9, are shown in Tables II through IX. Tables V and IX show the values obtained when Krilium was used as the only substrate.

The respiratory measurements of Agrobacterium tumefaciens respiring on the various substrates and in the presence of varying concentrations of Krilium are shown in Tables X to XVII, inclusively.

The above data for both organisms has been presented in graphical form in Figures 3 to 34.

TABLE II

Respiration of Rhizobium trifolii on Glucose in Presence of Krilium (Blend #6)

Concentrations of Krilium

Time in Minutes	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	16	13	17	14	16	-	15	18
15 - 30	28	23	32	27	26	-	27	29
30 - 45	38	32	45	39	42	-	43	45
45 - 60	49	41	56	48	55	-	59	61
60 - 75	62	53	69	59	70	-	72	75
75 - 90	71	60	80	69	81	-	85	88
B								
0 - 15	14	10	14	-	17	14	17	21
15 - 30	25	19	32	-	33	27	31	34
30 - 45	38	29	41	-	43	36	43	45
45 - 60	50	38	60	-	55	47	55	56
60 - 75	62	48	75	-	68	58	65	65
75 - 90	75	60	88	-	80	68	80	79
C								
0 - 15	13	-	17	13	15	14	16	-
15 - 30	24	-	32	27	32	28	29	-
30 - 45	35	-	47	39	45	42	43	-
45 - 60	51	-	59	49	59	54	60	-
60 - 75	65	-	73	60	72	66	73	-
75 - 90	75	-	88	74	83	76	98	-

TABLE III

Respiration of *Rhizobium trifolii* on Succinate in Presence of Krilium (Blend #6)

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	17	15	18	17	17	-	24	32
15 - 30	36	32	47	47	38	-	47	57
30 - 45	56	51	72	74	57	-	71	84
45 - 60	77	70	96	96	80	-	93	109
60 - 75	100	92	120	120	104	-	113	130
75 - 90	122	114	145	147	123	-	133	152
B								
0 - 15	22	-	16	19	24	27	21	32
15 - 30	49	-	36	41	46	49	47	61
30 - 45	70	-	58	63	69	74	72	89
45 - 60	92	-	79	85	90	97	97	118
60 - 75	115	-	103	111	109	114	120	143
75 - 90	135	-	122	129	129	136	142	170
C								
0 - 15	14	15	21	-	22	26	22	-
15 - 30	30	31	43	-	49	53	46	-
30 - 45	52	56	64	-	72	76	66	-
45 - 60	71	74	84	-	96	100	87	-
60 - 75	93	97	105	-	119	122	106	-
75 - 90	113	118	123	-	142	146	128	-

TABLE IV

Respiration of *Rhizobium trifolii* on Mannitol in Presence of Krilium (Blend #6)

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	9	-	11	9	11	-	12	14
15 - 30	16	-	19	17	18	-	23	25
30 - 45	22	-	24	21	22	-	37	37
45 - 60	29	-	28	23	28	-	51	51
60 - 75	33	-	33	25	33	-	65	64
75 - 90	38	-	39	27	38	-	79	77
B								
0 - 15	13	11	9	9	9	7	12	15
15 - 30	24	19	19	17	16	13	23	26
30 - 45	29	24	21	19	25	20	35	38
45 - 60	35	29	29	20	29	22	43	46
60 - 75	42	33	33	27	34	25	55	55
75 - 90	59	49	41	30	41	30	68	69
C								
0 - 15	11	11	9	-	11	9	13	-
15 - 30	18	16	17	-	17	15	24	-
30 - 45	25	24	24	-	22	17	33	-
45 - 60	31	28	32	-	28	21	47	-
60 - 75	38	32	38	-	33	22	56	-
75 - 90	42	36	45	-	39	26	70	-

TABLE V

Respiration of Rhizobium trifolii on Krilium (Blend #6) as the Substrate

Time in Minutes	Concentration of Krilium .05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	7	-	13	13	8	-
15 - 30	11	-	19	19	19	-
30 - 45	13	-	25	24	28	-
45 - 60	17	-	29	28	35	-
60 - 75	21	-	34	31	46	-
75 - 90	24	-	36	34	57	-
B						
0 - 15	12	9	8	-	11	14
15 - 30	17	15	15	-	20	27
30 - 45	21	19	22	-	25	29
45 - 60	25	23	30	-	36	39
60 - 75	28	24	33	-	43	45
75 - 90	32	28	37	-	52	52
C						
0 - 15	6	4	13	13	9	15
15 - 30	11	7	19	19	17	22
30 - 45	13	10	25	24	26	32
45 - 60	16	14	29	28	34	38
60 - 75	18	14	35	31	43	48
75 - 90	21	17	36	34	53	57

TABLE VI

Respiration of *Rhizobium trifolii* on Glucose in Presence of Krilium (Blend #9)

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	16	13	7	3	16	14	9	8
15 - 30	28	23	15	8	28	23	22	18
30 - 45	38	32	28	20	41	36	30	24
45 - 60	49	41	38	28	53	45	39	31
60 - 75	62	53	49	37	63	54	50	39
75 - 90	71	60	59	45	75	61	59	46
B								
0 - 15	14	10	14	-	11	10	13	-
15 - 30	25	19	26	-	21	21	22	-
30 - 45	38	29	37	-	33	31	34	-
45 - 60	50	38	47	-	44	40	43	-
60 - 75	62	48	58	-	52	49	54	-
75 - 90	75	60	67	-	65	62	66	-
C								
0 - 15	13	-	9	7	13	-	12	8
15 - 30	24	-	18	15	29	-	25	30
30 - 45	35	-	30	24	42	-	37	43
45 - 60	51	-	39	32	52	-	51	56
60 - 75	65	-	50	43	66	-	62	65
75 - 90	75	-	62	53	77	-	72	75

TABLE VII

Respiration of Rhizobium trifolii on Succinate in Presence of Krilium (Blend #9)

Concentrations of Krilium

Time in Minutes	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	17	15	21	24	21	22	11	-
15 - 30	36	32	41	41	37	37	25	-
30 - 45	56	51	59	59	51	45	41	-
45 - 60	77	70	76	76	67	60	58	-
60 - 75	100	92	95	94	80	70	76	-
75 - 90	122	114	113	113	95	85	92	-
B								
0 - 15	22	-	21	-	21	23	20	25
15 - 30	49	-	38	-	45	47	40	50
30 - 45	70	-	54	-	67	69	63	74
45 - 60	92	-	69	-	89	92	84	97
60 - 75	115	-	83	-	114	117	114	130
75 - 90	135	-	96	-	138	140	126	143
C								
0 - 15	14	15	21	25	-	-	-	-
15 - 30	30	31	43	45	-	-	-	-
30 - 45	52	56	65	68	-	-	-	-
45 - 60	71	74	85	87	-	-	-	-
60 - 75	93	97	107	108	-	-	-	-
75 - 90	113	118	130	132	-	-	-	-

TABLE VIII

Respiration of Rhizobium trifolii on Mannitol in Presence of Krilium (Blend #9)

Concentrations of Krilium

Time in Minutes	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	9	-	8	7	8	6	15	16
15 - 30	16	-	15	10	13	10	21	24
30 - 45	22	-	19	16	17	13	28	31
45 - 60	29	-	25	19	21	16	32	34
60 - 75	33	-	29	21	25	21	40	41
B								
0 - 15	13	11	13	7	8	3	11	13
15 - 30	24	19	20	13	12	5	16	21
30 - 45	29	24	26	17	19	10	21	27
45 - 60	35	29	31	21	23	11	26	31
60 - 75	42	33	37	23	27	12	29	34
75 - 90	59	49	42	26	31	14	33	37
C								
0 - 15	11	11	-	-	-	-	-	-
15 - 30	18	16	-	-	-	-	-	-
30 - 45	25	24	-	-	-	-	-	-
45 - 60	31	28	-	-	-	-	-	-
60 - 75	38	32	-	-	-	-	-	-
75 - 90	42	36	-	-	-	-	-	-

TABLE X

Respiration of Agrobacterium tumefaciens on Glucose in Presence of Krillium (Blend #6)

Time in Minutes	Concentrations of Krillium 0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	-	-	-	-	-	-	-	-
15 - 30	29	21	26	18	29	22	30	29
30 - 45	-	-	-	-	-	-	-	-
45 - 60	56	46	56	43	59	60	61	56
60 - 75	-	-	-	-	-	-	-	-
75 - 90	87	69	68	69	90	74	95	87
B								
0 - 15	-	-	12	7	16	10	13	14
15 - 30	26	-	25	15	24	15	26	27
30 - 45	-	-	38	27	37	26	46	46
45 - 60	58	-	52	39	49	36	58	58
60 - 75	-	-	64	50	60	44	75	74
75 - 90	90	-	80	60	71	52	88	86
C								
0 - 15	-	-	11	-	9	-	-	-
15 - 30	24	-	26	-	25	-	-	-
30 - 45	-	-	37	-	38	-	-	-
45 - 60	60	-	47	-	54	-	-	-
60 - 75	-	-	58	-	69	-	-	-
75 - 90	90	-	65	-	85	-	-	-

TABLE XI

Respiration of *Agrobacterium tumefaciens* on Succinate in Presence of Krilium (Blend #6)

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	-	-	-	-	20	26	24	32
15 - 30	44	53	48	53	44	52	44	57
30 - 45	-	-	-	-	69	80	67	82
45 - 60	87	101	95	103	93	107	91	112
60 - 75	-	-	-	-	113	128	113	136
75 - 90	126	144	136	155	137	154	136	161
B								
0 - 15	24	-	17	24	16	24	9	17
15 - 30	43	-	36	44	44	53	28	39
30 - 45	68	-	55	65	65	76	51	65
45 - 60	94	-	76	90	85	100	72	102
60 - 75	117	-	97	120	107	124	93	114
75 - 90	139	-	125	139	126	146	110	142
C								
0 - 15	-	-	15	-	16	17	16	23
15 - 30	-	-	40	-	36	39	35	45
30 - 45	-	-	63	-	56	60	56	67
45 - 60	-	-	83	-	75	81	76	87
60 - 75	-	-	105	-	92	106	96	108
75 - 90	-	-	124	-	107	123	111	126

TABLE XII

Respiration of Agrobacterium tumefaciens on Mannitol in Presence of Krilium (Blend #6)

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	-	-	-	-	-	-	11	11
15 - 30	29	-	40	21	30	19	24	22
30 - 45	-	-	-	-	-	-	38	34
45 - 60	64	-	75	39	66	48	51	46
60 - 75	-	-	-	-	-	-	68	61
75 - 90	106	-	107	60	105	82	83	73
B								
0 - 15	-	-	8	3	-	-	7	-
15 - 30	34	-	18	11	32	27	16	-
30 - 45	-	-	26	17	-	-	29	-
45 - 60	68	-	37	27	65	52	45	-
60 - 75	-	-	50	34	-	-	58	-
75 - 90	105	-	62	44	103	80	75	-
C								
0 - 15	-	-	27	-	11	10	11	14
15 - 30	-	-	45	-	23	21	19	23
30 - 45	-	-	63	-	31	28	31	33
45 - 60	-	-	84	-	41	37	40	41
60 - 75	-	-	95	-	53	46	52	52
75 - 90	-	-	115	-	67	55	64	62

TABLE XIII

Respiration of Agrobacterium tumefaciens on Krilium (Blend #6) as the Substrate

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	-	-	14	14	9	6	14	-
15 - 30	-	-	19	21	11	7	20	-
30 - 45	-	-	23	23	16	11	33	-
45 - 60	-	-	29	28	20	13	45	-
60 - 75	-	-	29	28	22	16	54	-
75 - 90	-	-	33	30	26	20	64	-
B								
0 - 15	-	-	7	-	9	-	11	10
15 - 30	-	-	12	-	18	-	24	25
30 - 45	-	-	13	-	21	-	36	34
45 - 60	-	-	16	-	25	-	47	45
60 - 75	-	-	18	-	29	-	60	55
75 - 90	-	-	21	-	33	-	73	67
C								
0 - 15	-	-	7	-	-	-	12	16
15 - 30	-	-	13	-	-	-	25	27
30 - 45	-	-	14	-	-	-	36	37
45 - 60	-	-	15	-	-	-	46	47
60- 75	-	-	20	-	-	-	55	54
75 - 90	-	-	25	-	-	-	65	64

TABLE XIV

Respiration of Agrobacterium tumefaciens on Glucose in Presence of Krilium (Blend #9)

Time in Minutes	Concentrations of Krilium 0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	-	-	14	11	-	-	-	-
15 - 30	29	21	28	21	25	22	30	25
30 - 45	-	-	39	32	-	-	-	-
45 - 60	56	46	50	39	61	53	54	43
60 - 75	-	-	63	50	-	-	-	-
75 - 90	87	69	75	61	96	82	77	59
B								
0 - 15	-	-	13	-	11	-	-	-
15 - 30	26	-	22	-	22	-	36	-
30 - 45	-	-	33	-	35	-	-	-
45 - 60	58	-	43	-	49	-	73	-
60 - 75	-	-	60	-	59	-	-	-
75 - 90	90	-	71	-	71	-	98	-
C								
0 - 15	-	-	-	-	-	-	11	9
15 - 30	24	-	-	-	23	-	21	17
30 - 45	-	-	-	-	-	-	26	19
45 - 60	60	-	-	-	57	-	42	34
60 - 75	-	-	-	-	-	-	56	44
75 - 90	97	-	-	-	93	-	68	54

TABLE XV

Respiration of Agrobacterium tumefaciens on Succinate in Presence of Krilium (Blend #9)

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	-	-	17	21	21	-	-	-
15 - 30	44	53	42	50	43	-	41	32
30 - 45	-	-	64	73	67	-	-	-
45 - 60	87	101	85	97	89	-	82	68
60 - 75	-	-	102	115	110	-	-	-
75 - 90	126	144	121	138	135	-	119	103
B								
0 - 15	24	-	12	-	22	-	16	-
15 - 30	43	-	28	-	43	-	30	-
30 - 45	68	-	46	-	71	-	50	-
45 - 60	94	-	66	-	89	-	66	-
60 - 75	117	-	81	-	110	-	82	-
75 - 90	139	-	110	-	129	-	99	-
C								
0 - 15	-	-	-	-	-	-	15	19
15 - 30	-	-	-	-	-	-	31	38
30 - 45	-	-	-	-	-	-	48	58
45 - 60	-	-	-	-	-	-	63	77
60 - 75	-	-	-	-	-	-	79	94
75 - 90	-	-	-	-	-	-	92	103

TABLE XVI

Respiration of Agrobacterium tumefaciens on Mannitol in Presence of Krilium (Blend #9)

Time in Minutes	Concentrations of Krilium							
	0.0%		.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	-	-	8	8	7	7	9	9
15 - 30	29	-	17	14	13	11	17	16
30 - 45	-	-	28	23	18	16	25	22
45 - 60	64	-	37	29	25	22	35	28
60 - 75	-	-	47	36	31	27	41	32
75 - 90	106	-	61	46	39	36	49	38
B								
0 - 15	-	-	7	6	6	4	9	8
15 - 30	34	-	12	11	12	7	19	16
30 - 45	-	-	19	17	18	11	28	22
45 - 60	68	-	27	23	26	16	37	29
60 - 75	-	-	35	29	36	21	47	34
75 - 90	105	-	43	36	45	29	53	39
C								
0 - 15	-	-	3	-	6	-	-	-
15 - 30	-	-	8	-	15	-	-	-
30 - 45	-	-	13	-	22	-	-	-
45 - 60	-	-	21	-	31	-	-	-
60 - 75	-	-	31	-	41	-	-	-
75 - 90	-	-	42	-	53	-	-	-

TABLE XVII

Respiration of Agrobacterium tumefaciens on Krilium (Blend #9) as the Substrate

Time in Minutes	Concentrations of Krilium					
	.05%		.10%		1.0%	
A	O_2	CO_2	O_2	CO_2	O_2	CO_2
0 - 15	5	8	4	5	9	12
15 - 30	11	13	8	9	16	18
30 - 45	12	16	11	11	23	26
45 - 60	16	18	13	14	25	29
60 - 75	21	23	16	15	31	30
75 - 90	24	25	19	18	35	37
B						
0 - 15	9	-	-	-	6	9
15 - 30	13	-	14	-	12	12
30 - 45	16	-	-	-	20	20
45 - 60	20	-	25	-	25	22
60 - 75	25	-	-	-	28	30
75 - 90	28	-	33	-	33	34
C						
0 - 15	-	-	3	11	-	-
15 - 30	7	-	11	20	-	-
30 - 45	9	-	13	21	-	-
45 - 60	14	-	17	24	-	-
60 - 75	17	-	20	26	-	-
75 - 90	21	-	24	30	-	-

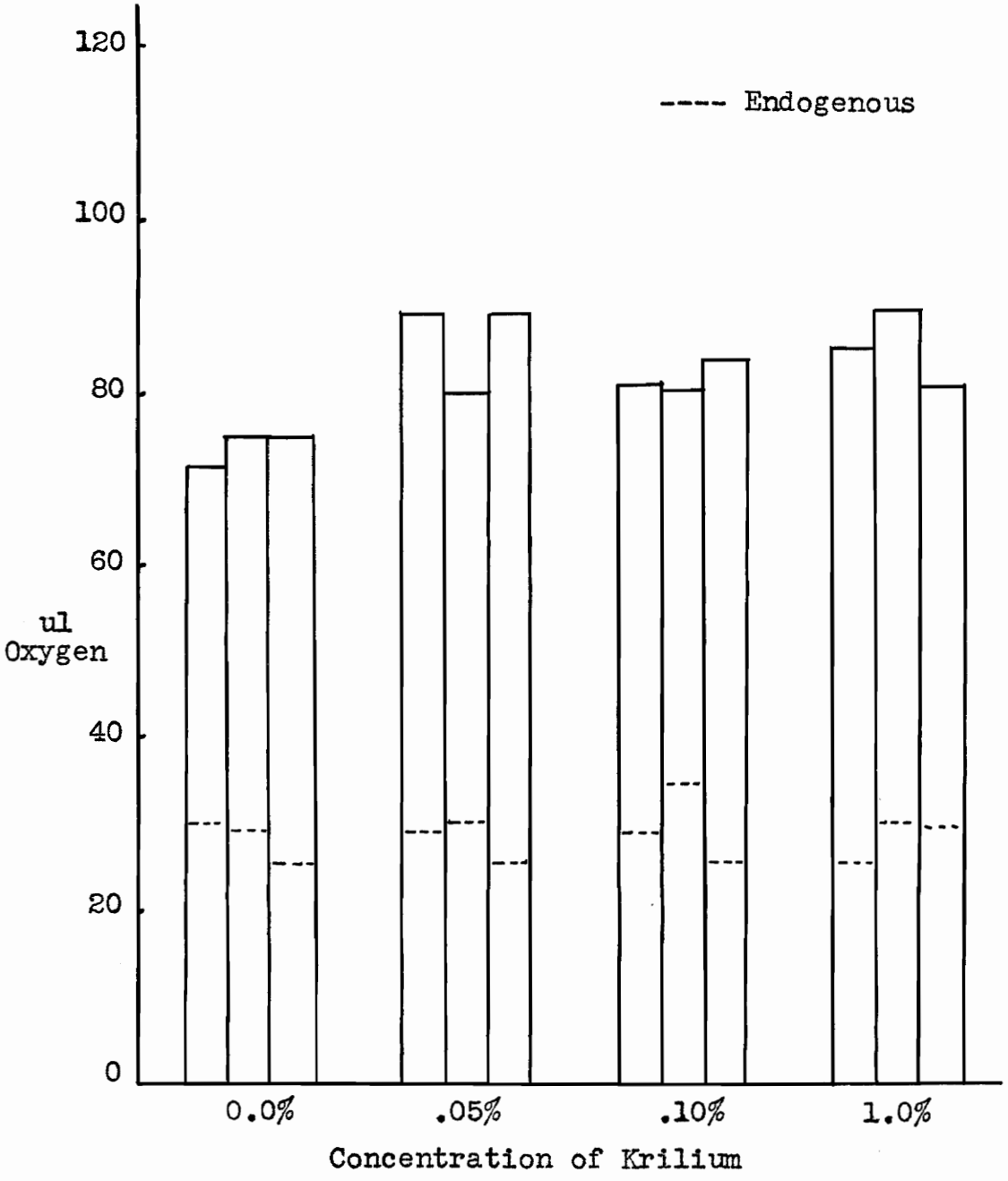

Figure 3. Oxygen uptake of Rhizobium trifolii 205 on .02 M Glucose in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

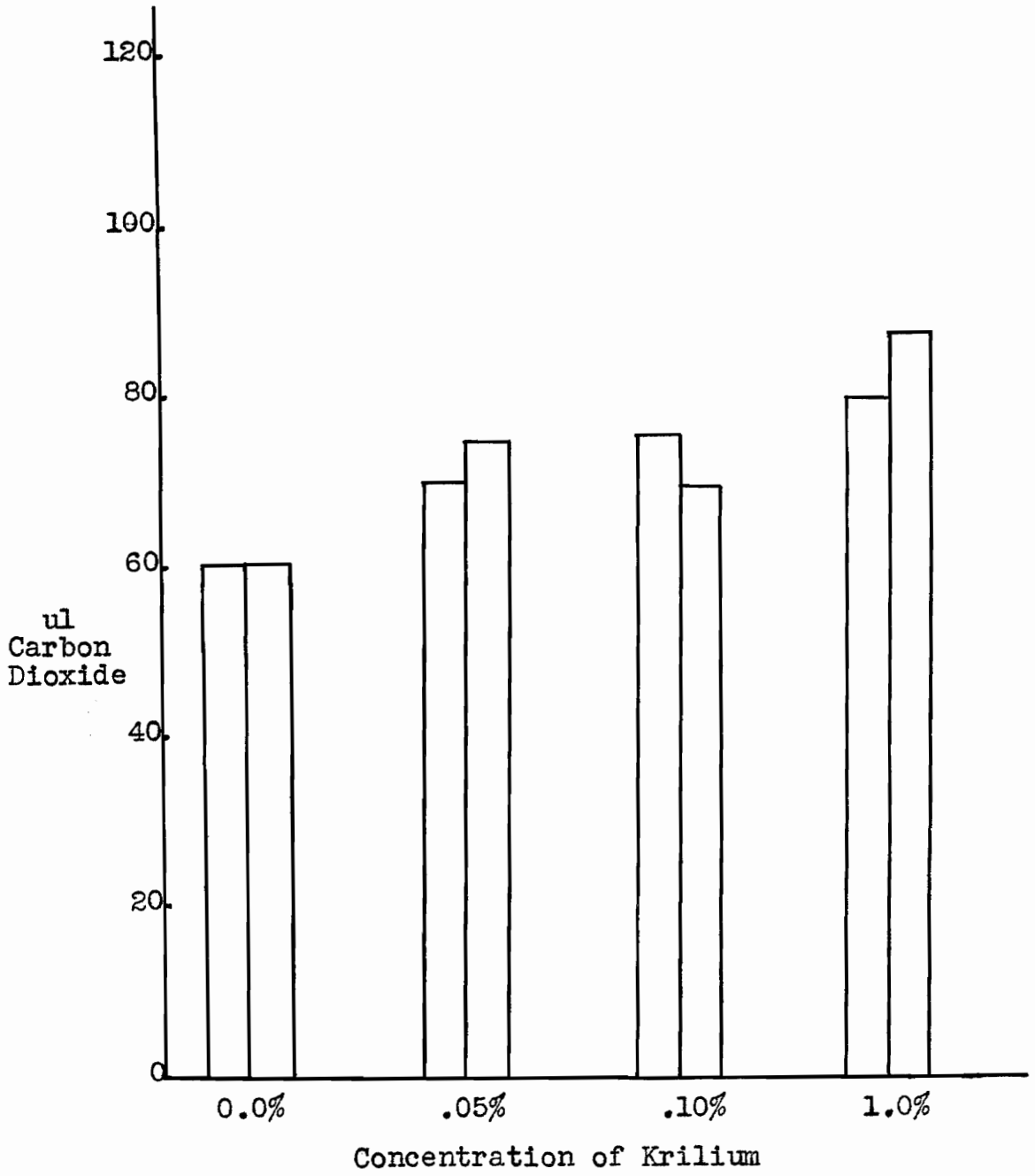

Figure 4. Carbon dioxide liberated by Rhizobium trifolii 205 on .02 M Glucose in presence of shown concentrations of Krilium (Blend #6). 1.5 mg.cells Time is 90 minutes.

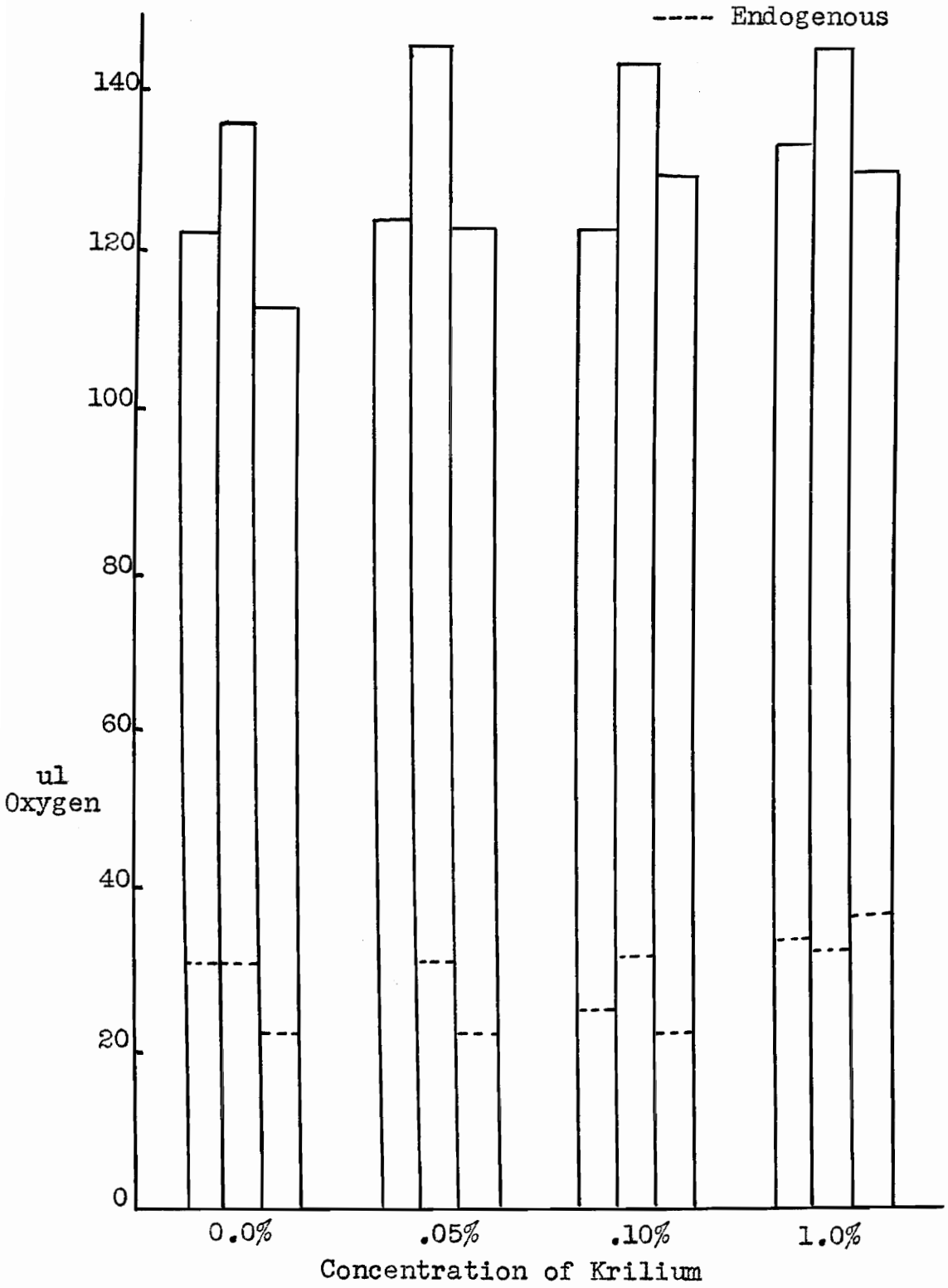

Figure 5. Oxygen uptake of Rhizobium trifolii 205 on .02 M Succinate in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

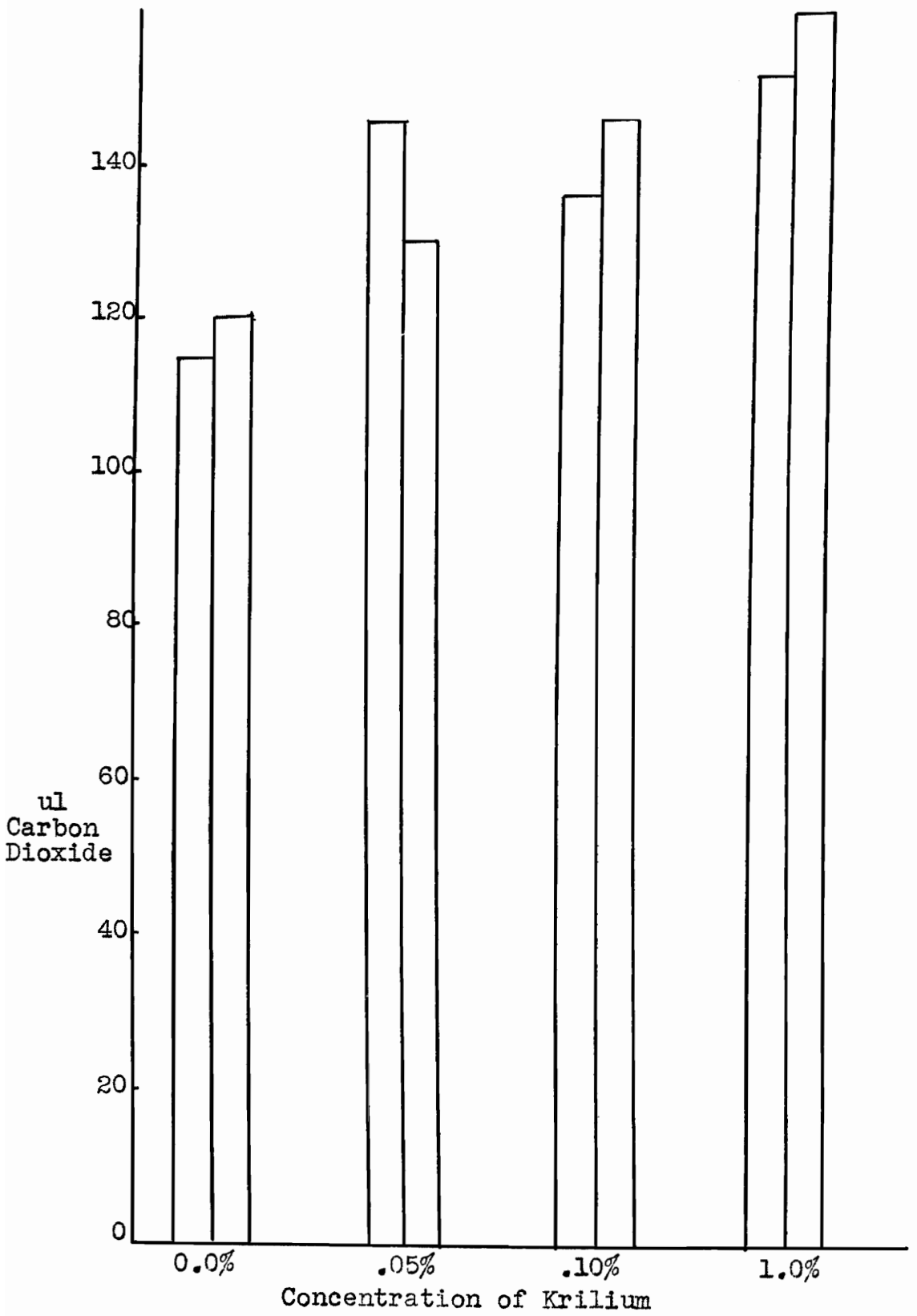

Figure 6. Carbon dioxide liberated by Rhizobium trifolii 205 on .02 M Succinate in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

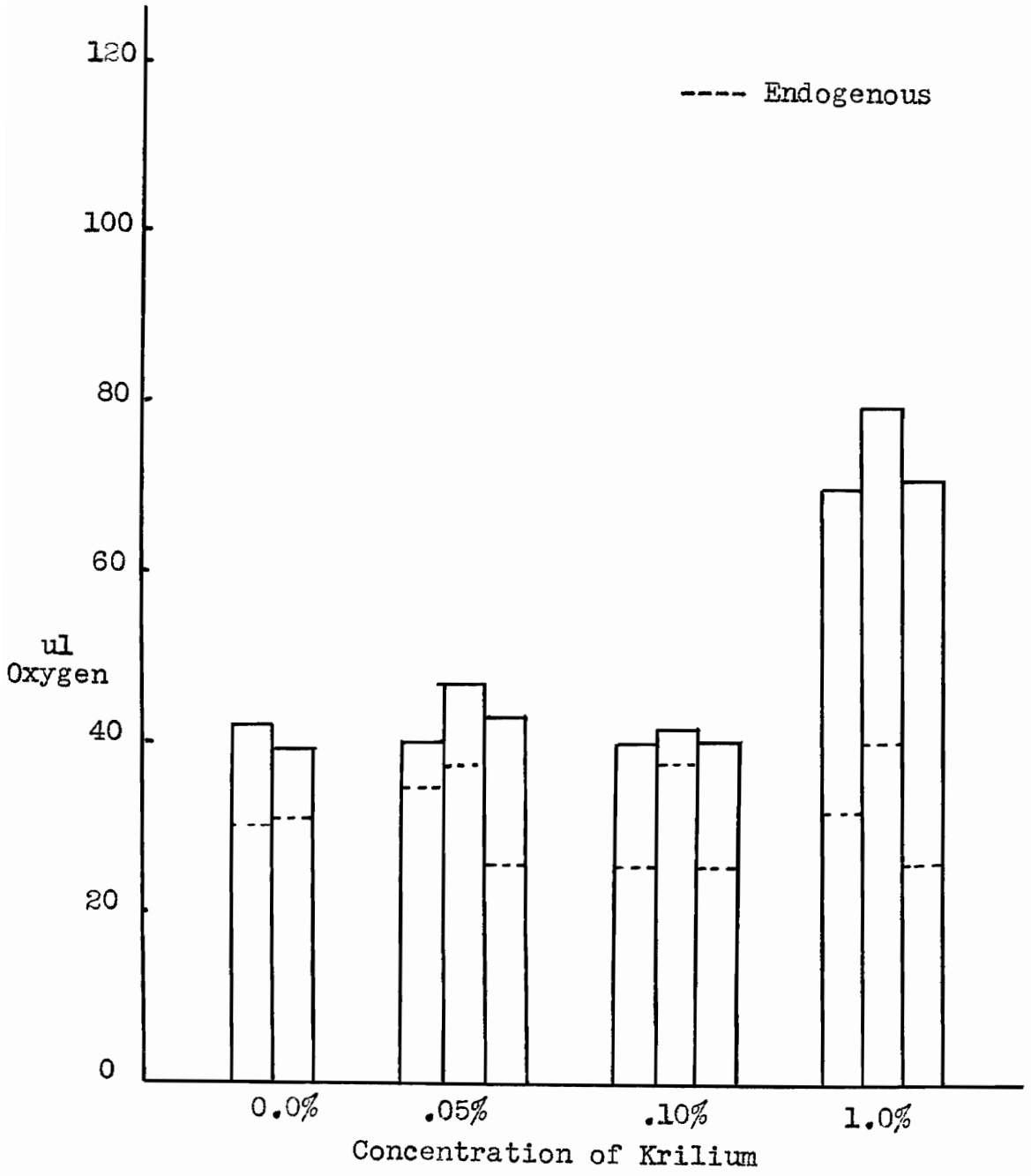

Figure 7. Oxygen uptake of Rhizobium trifolii 205 on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

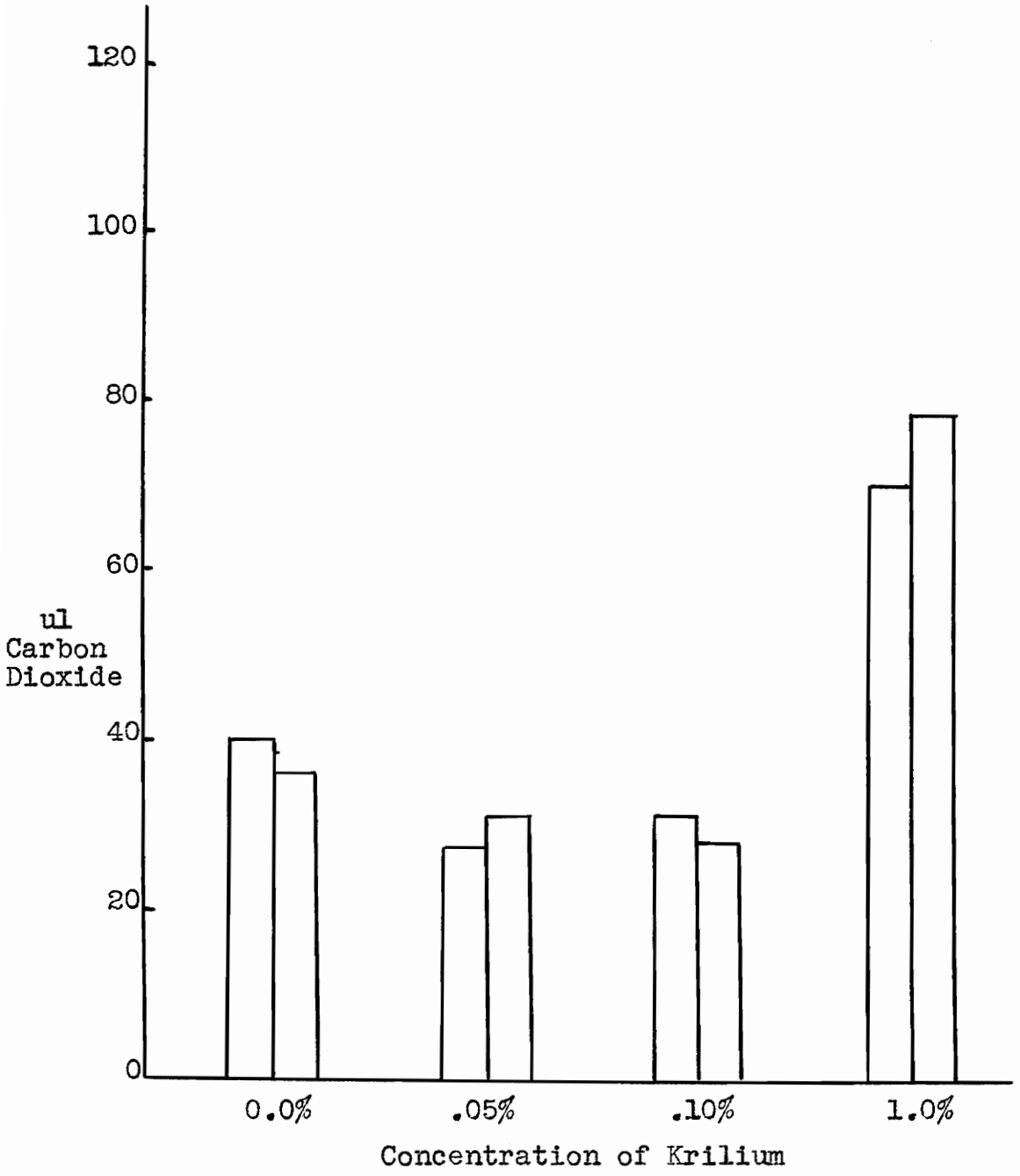

Figure 8. Carbon dioxide liberated by Rhizobium trifolii 205 on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells Time is 90 minutes.

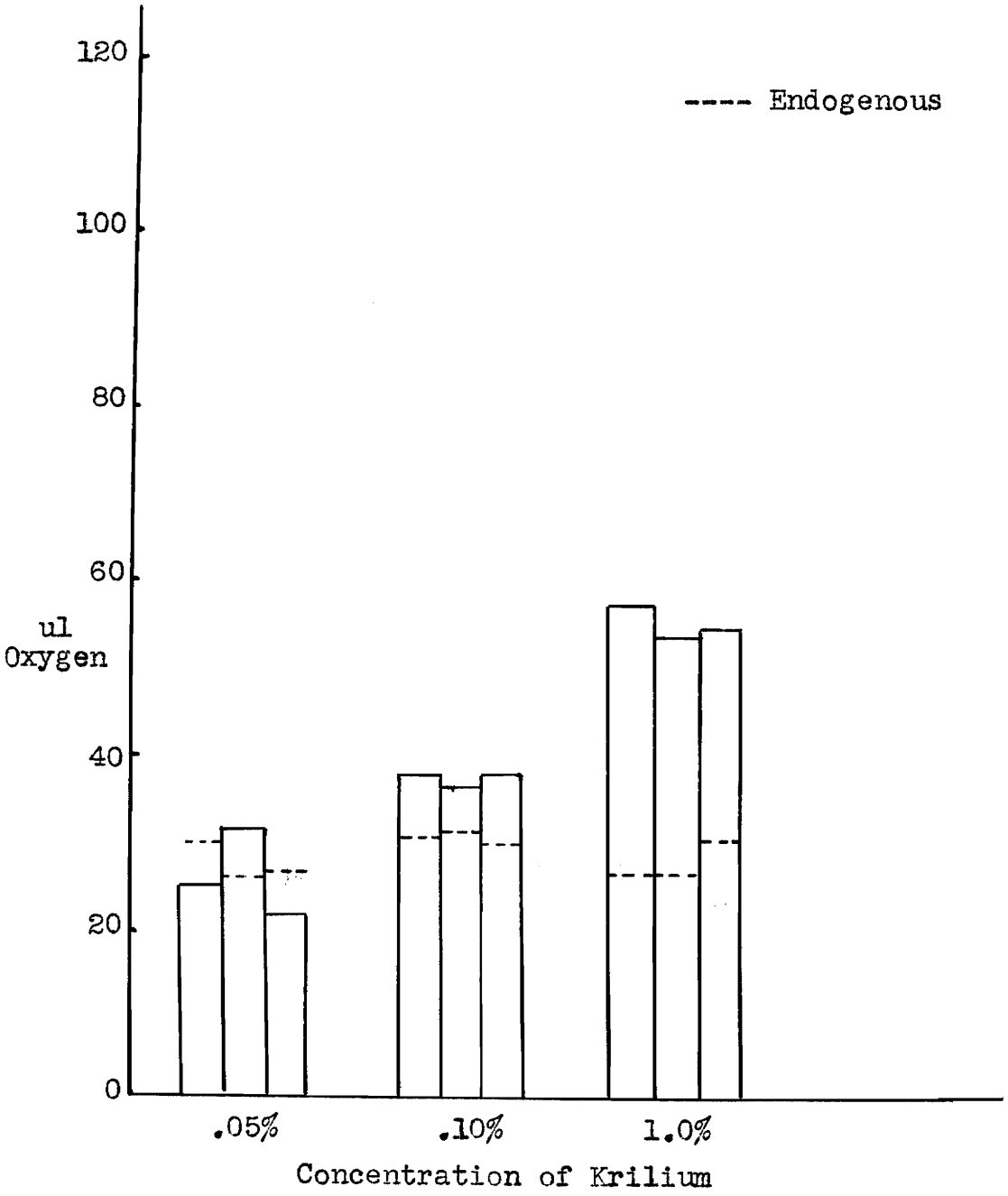

Figure 9. Oxygen uptake of Rhizobium trifolii 205 on shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

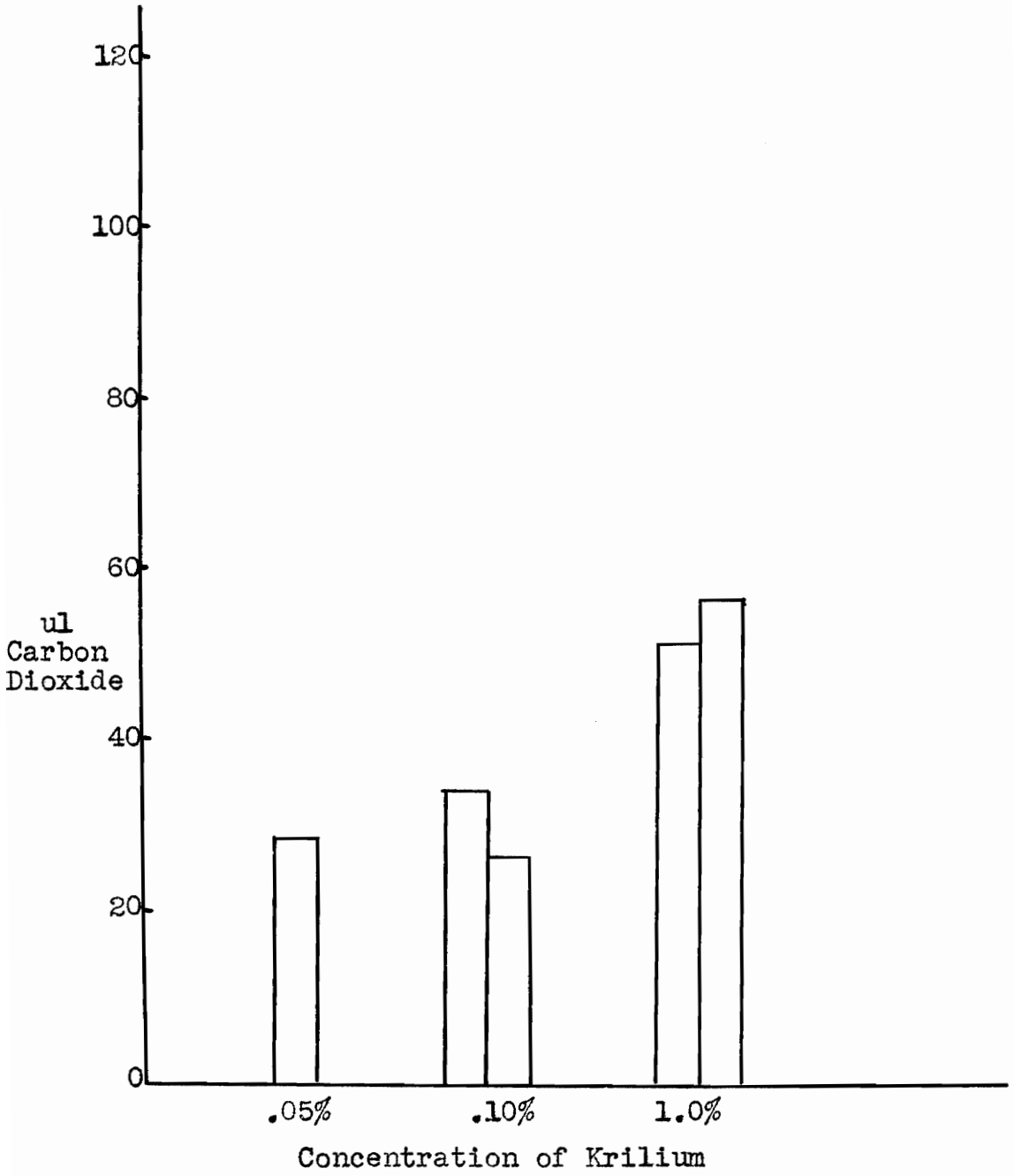

Figure 10. Carbon dioxide liberated by Rhizobium trifolii 205 on shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

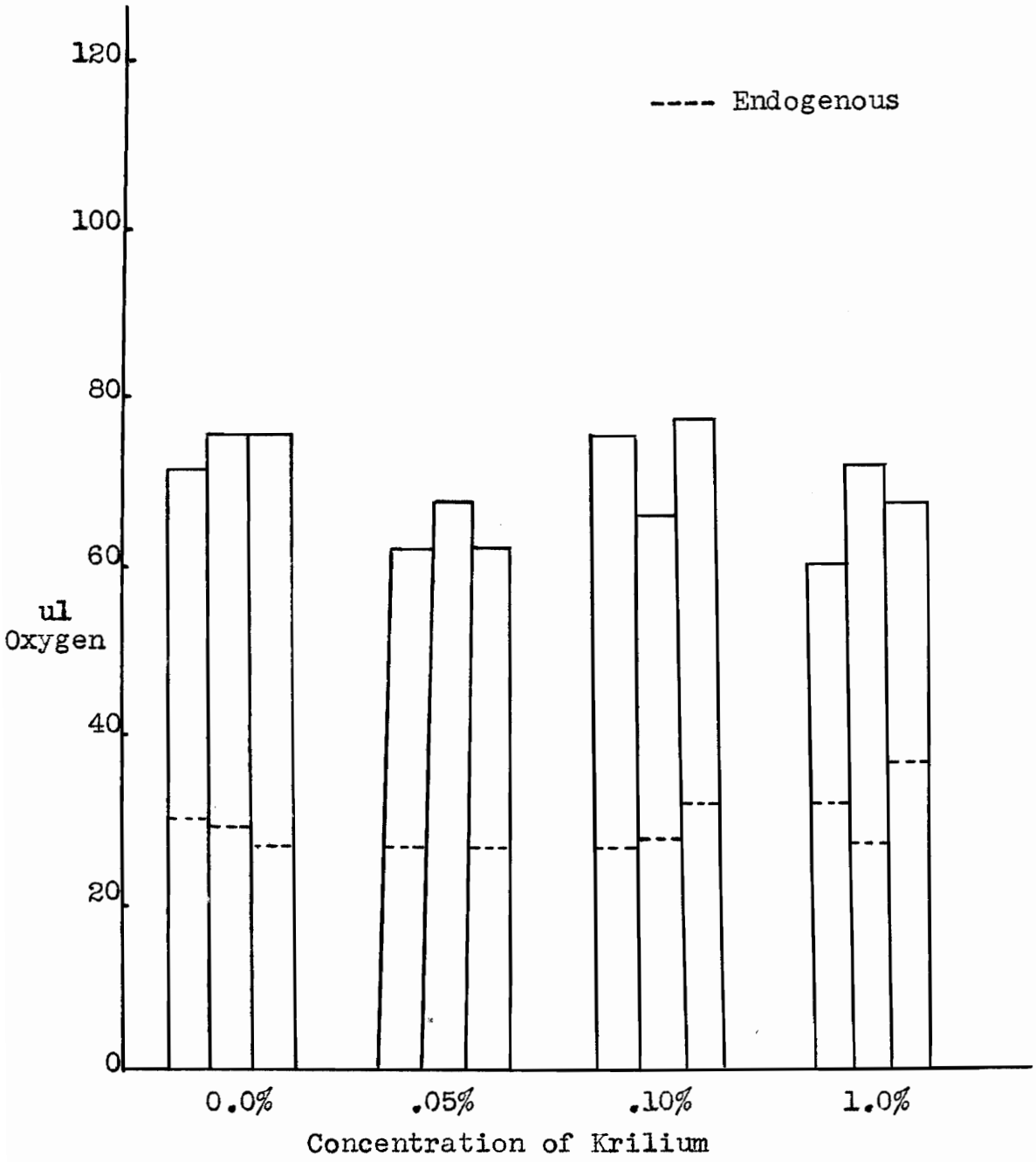

Figure 11. Oxygen uptake of Rhizobium trifolii 205 on .02 M Glucose in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

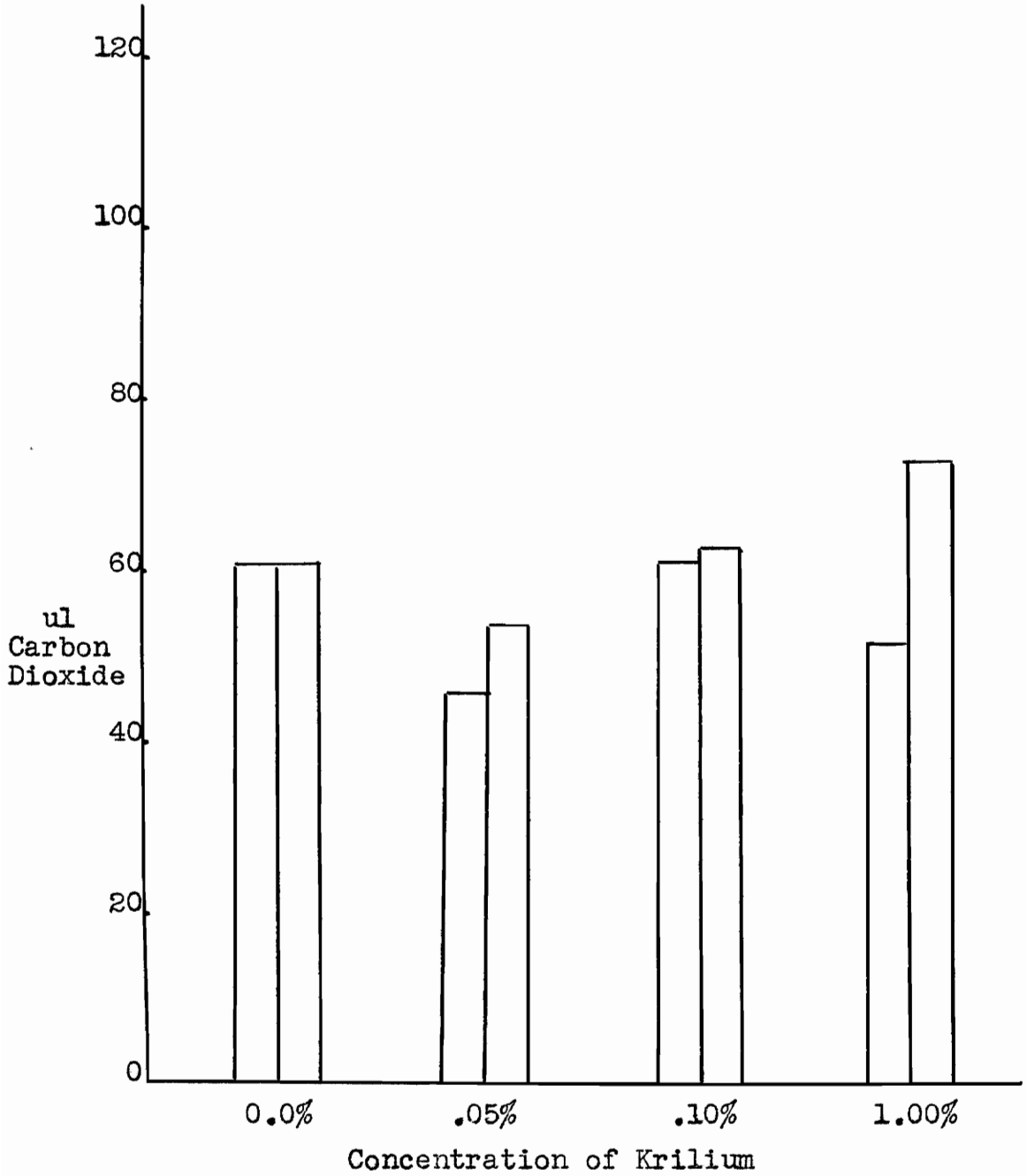

Figure 12. Carbon dioxide liberated by Rhizobium trifolii 205 on .02 M Glucose in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells Time is 90 minutes.

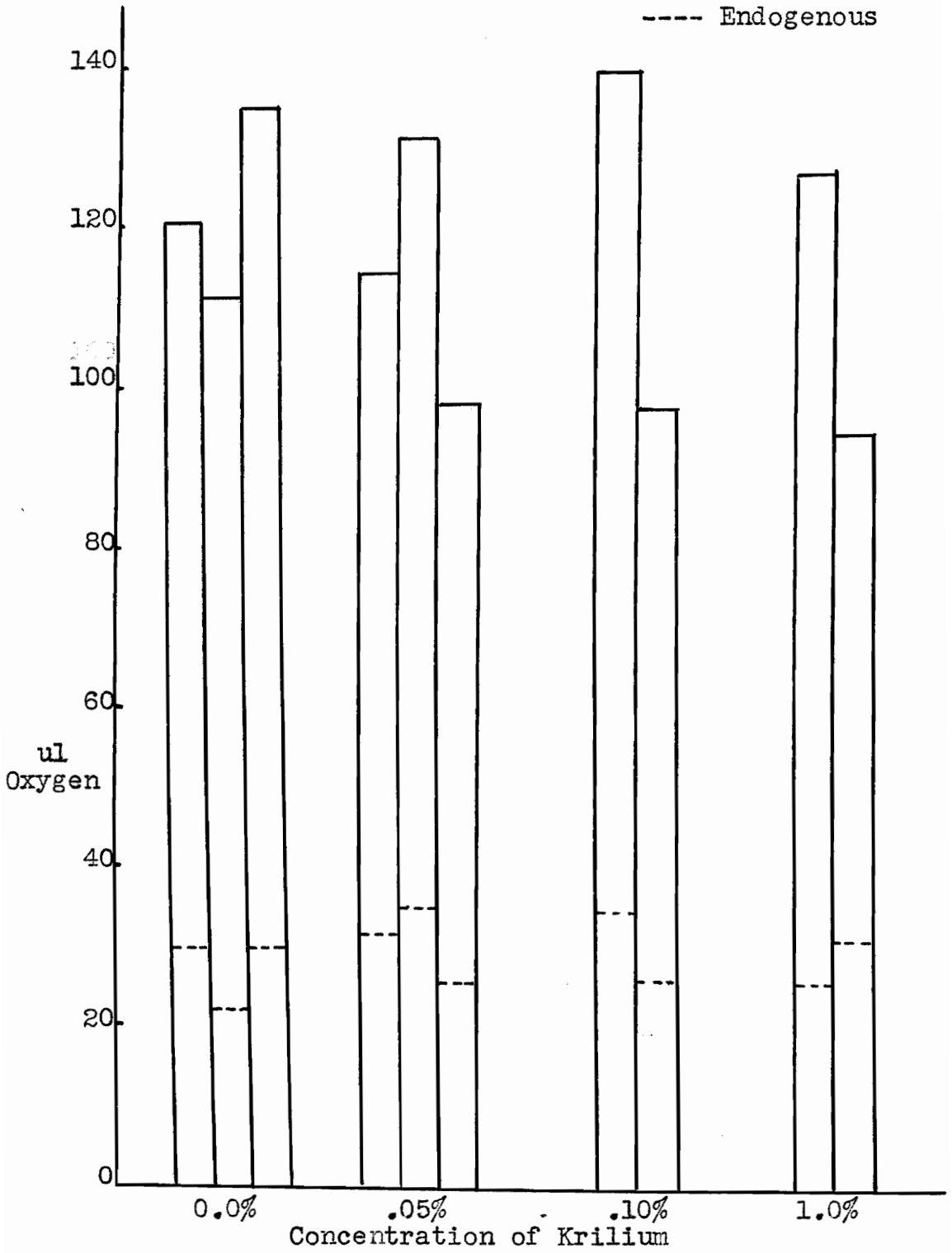

Figure 13. Oxygen uptake of Rhizobium trifolii 205 on .02 M Succinate in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells. Time is 90 minutes.

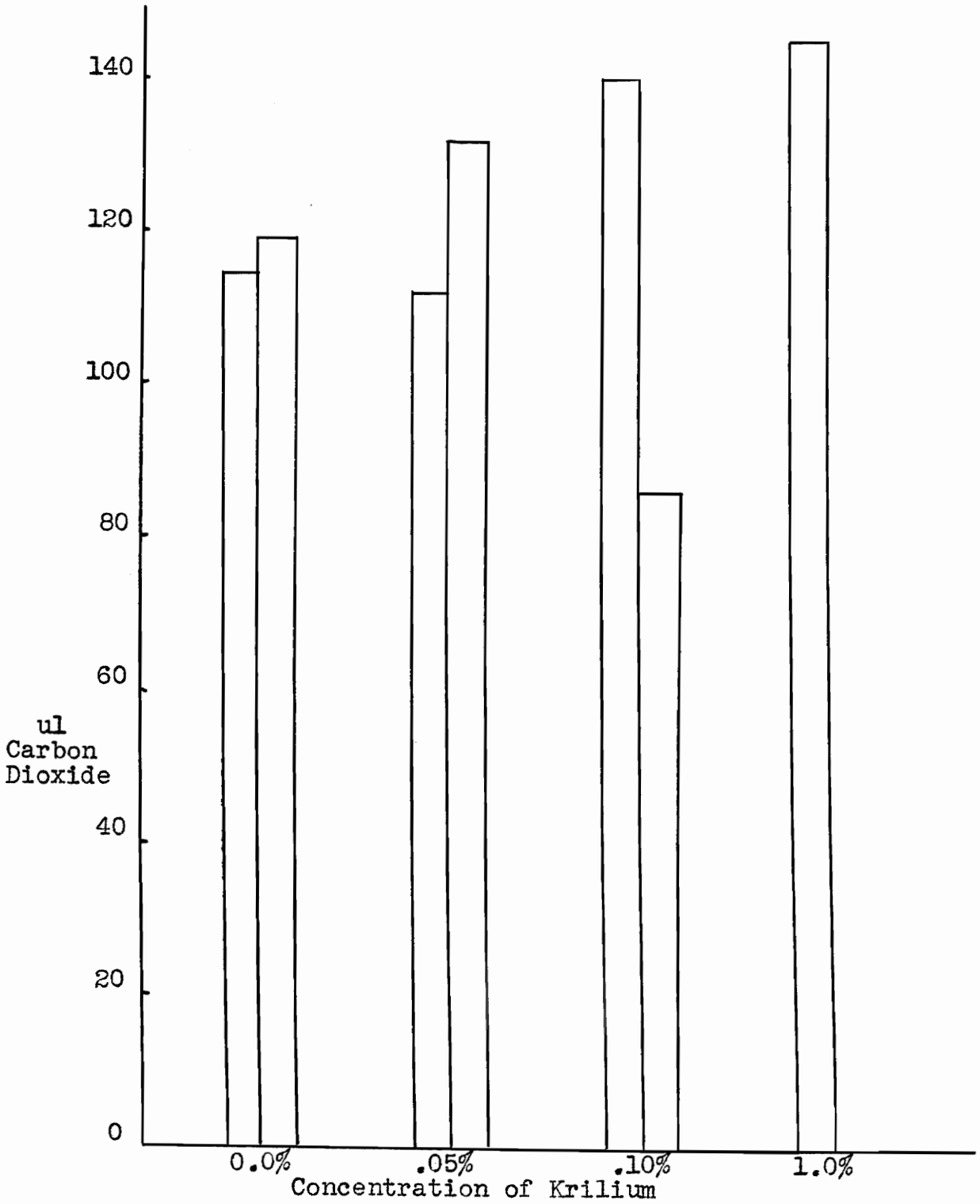

Figure 14. Carbon dioxide liberated by Rhizobium trifolii 205 on .02 M Succinate in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

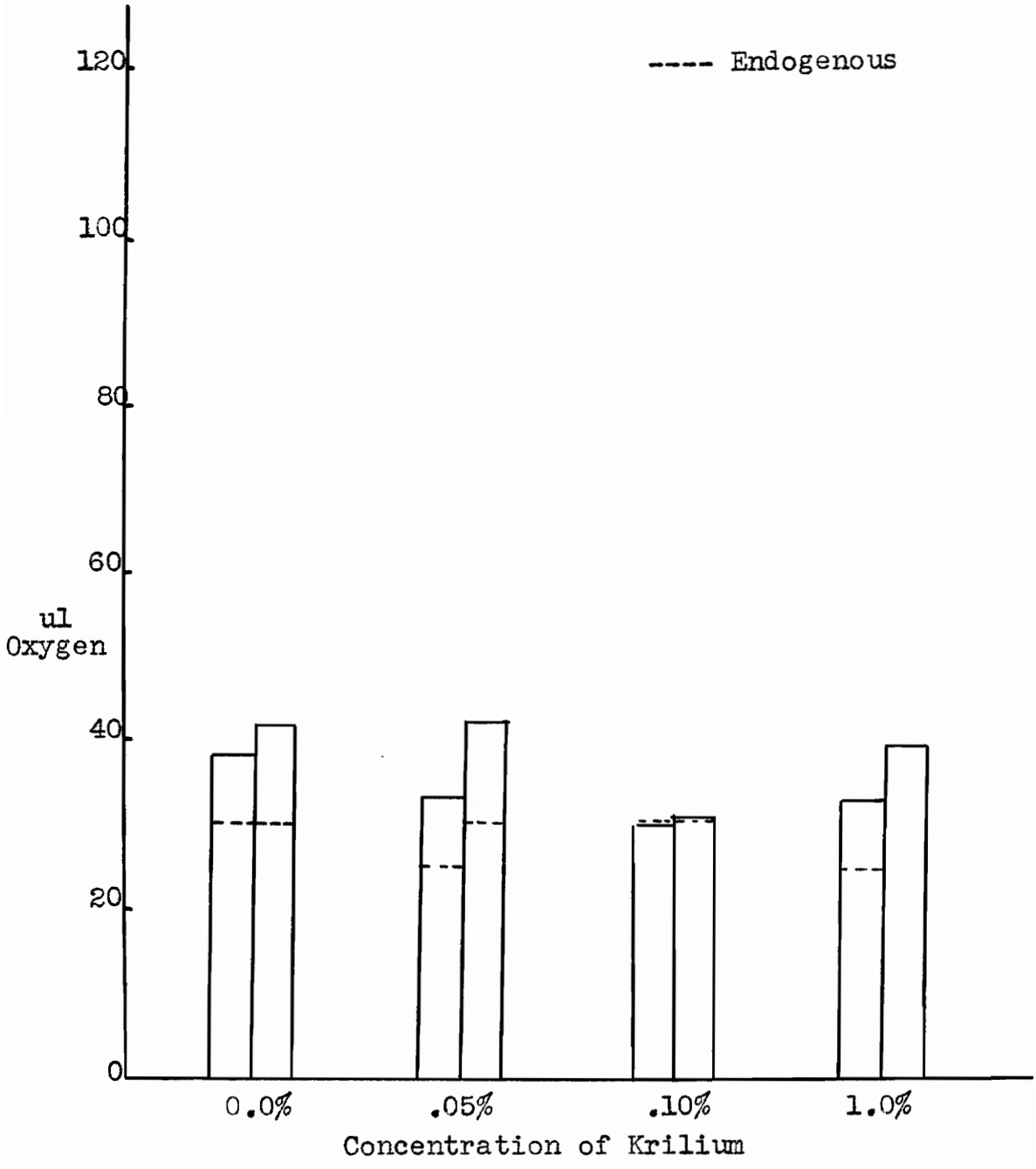

Figure 15. Oxygen uptake of Rhizobium trifolii 205 on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

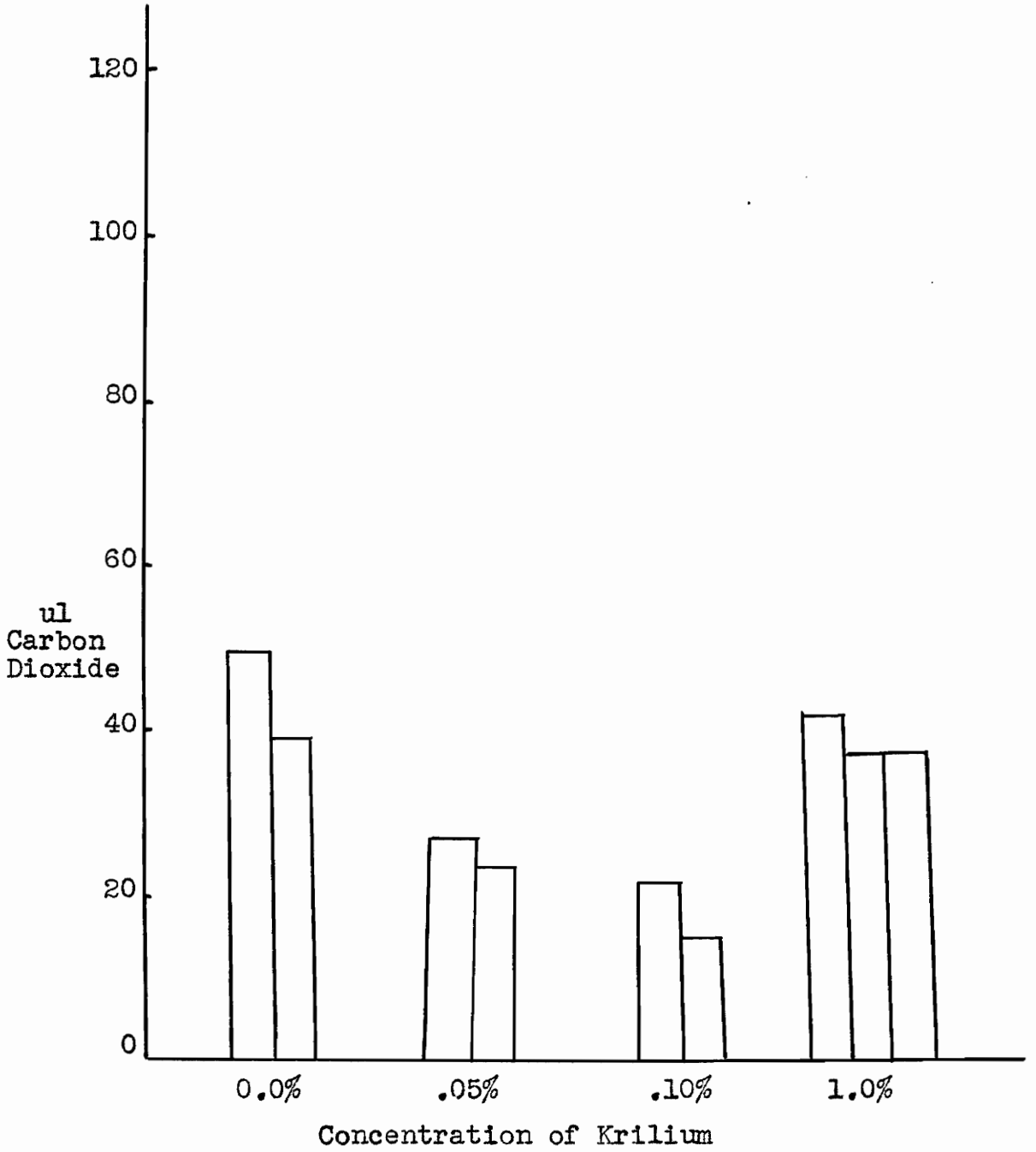

Figure 16. Carbon dioxide liberated by Rhizobium trifolii 205 on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

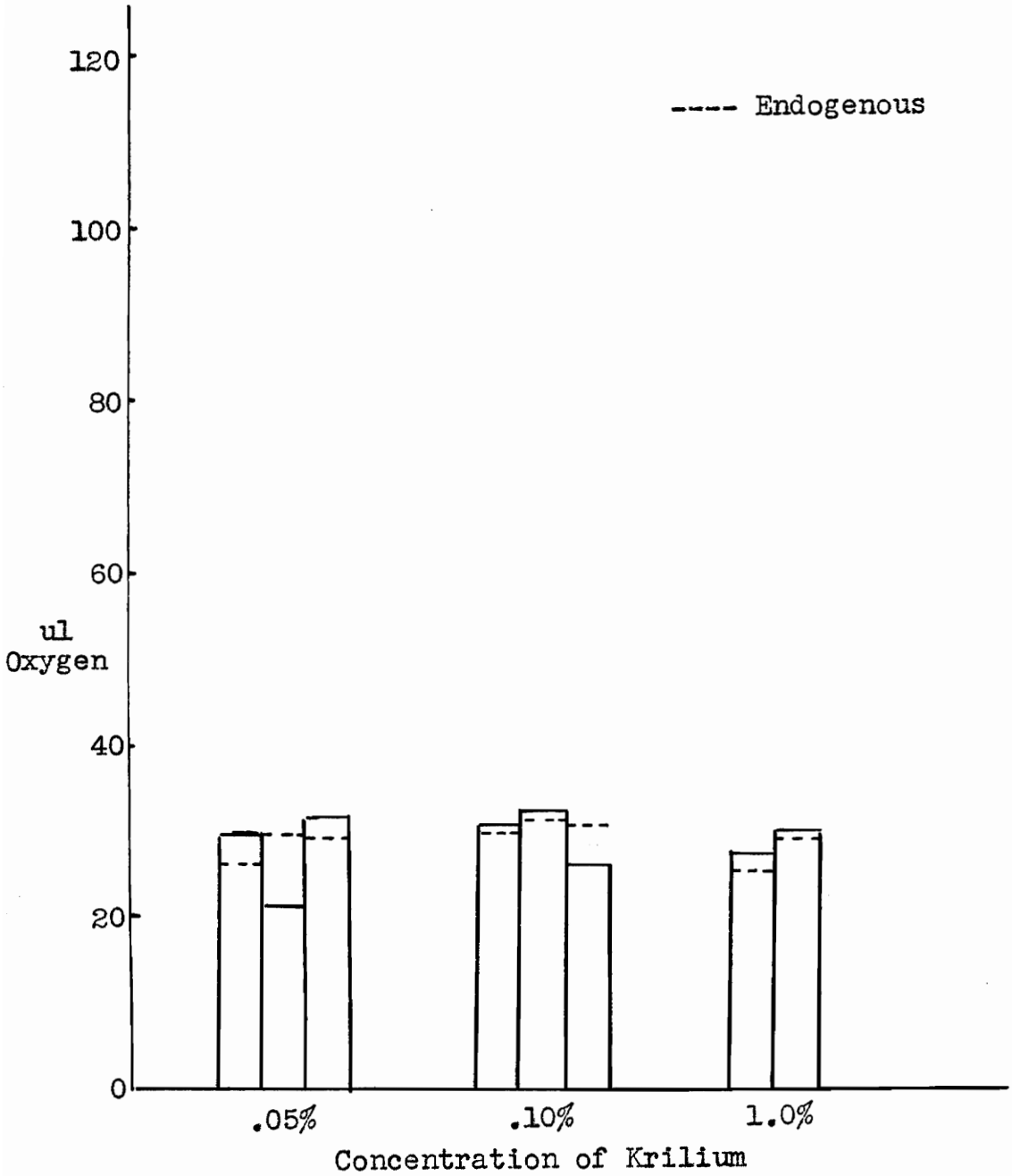

Figure 17. Oxygen uptake of Rhizobium trifolii 205 on shown concentrations of Krilium (Blend #9). 1.5 mg. cells Time is 90 minutes.

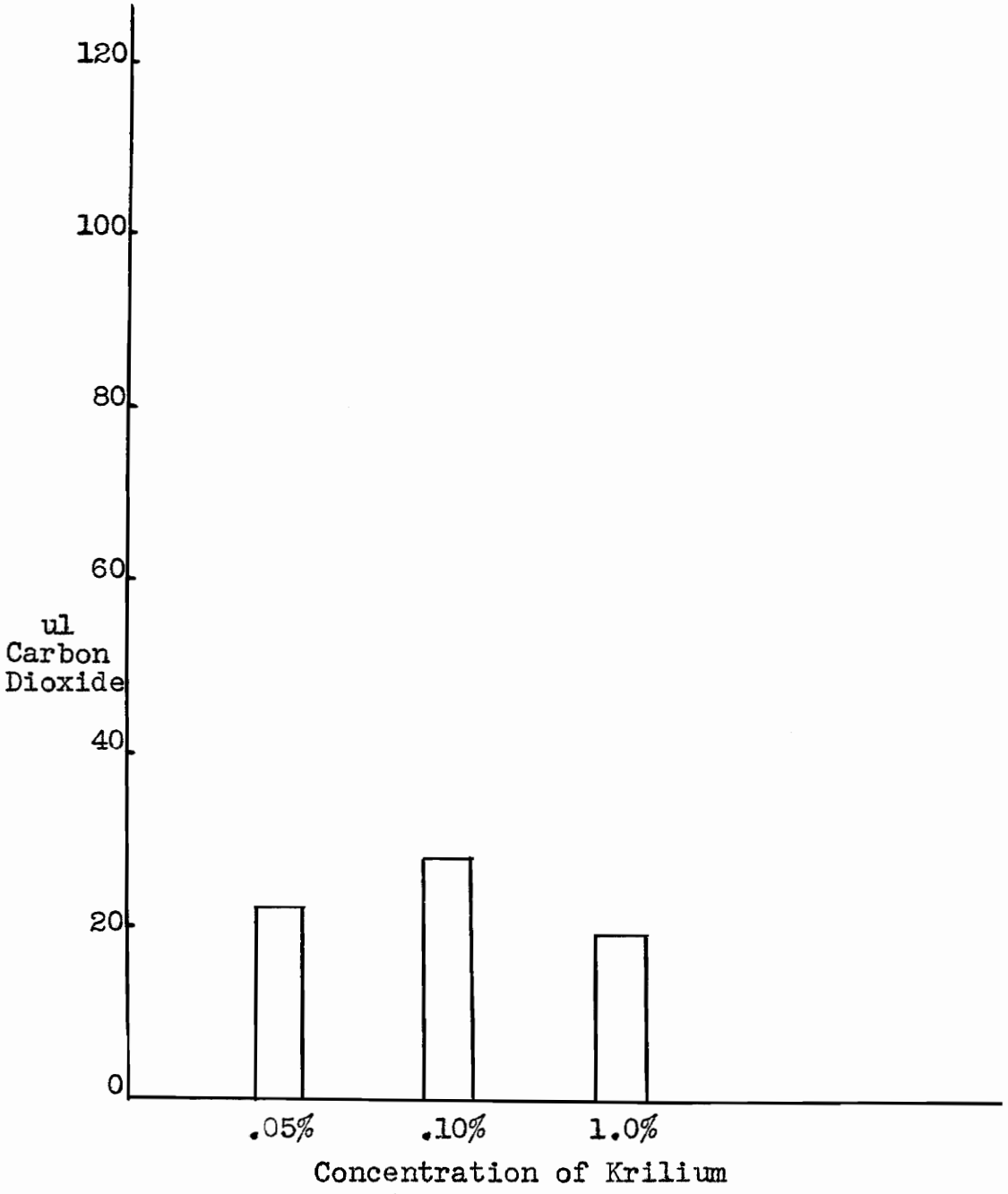

Figure 18. Carbon dioxide liberated by Rhizobium trifolii 205 on shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

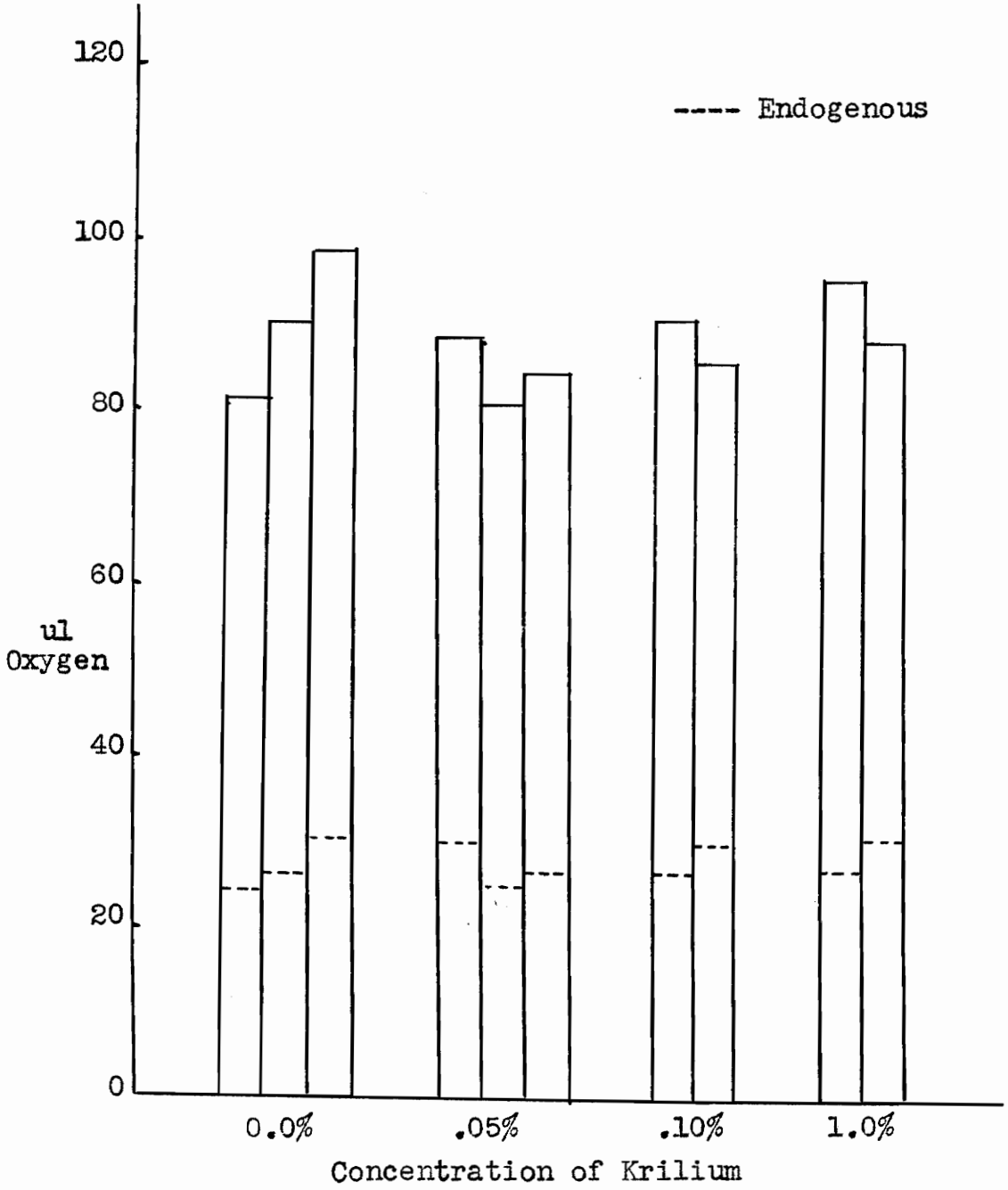

Figure 19. Oxygen uptake of Agrobacterium tumefaciens on .02 M Glucose in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

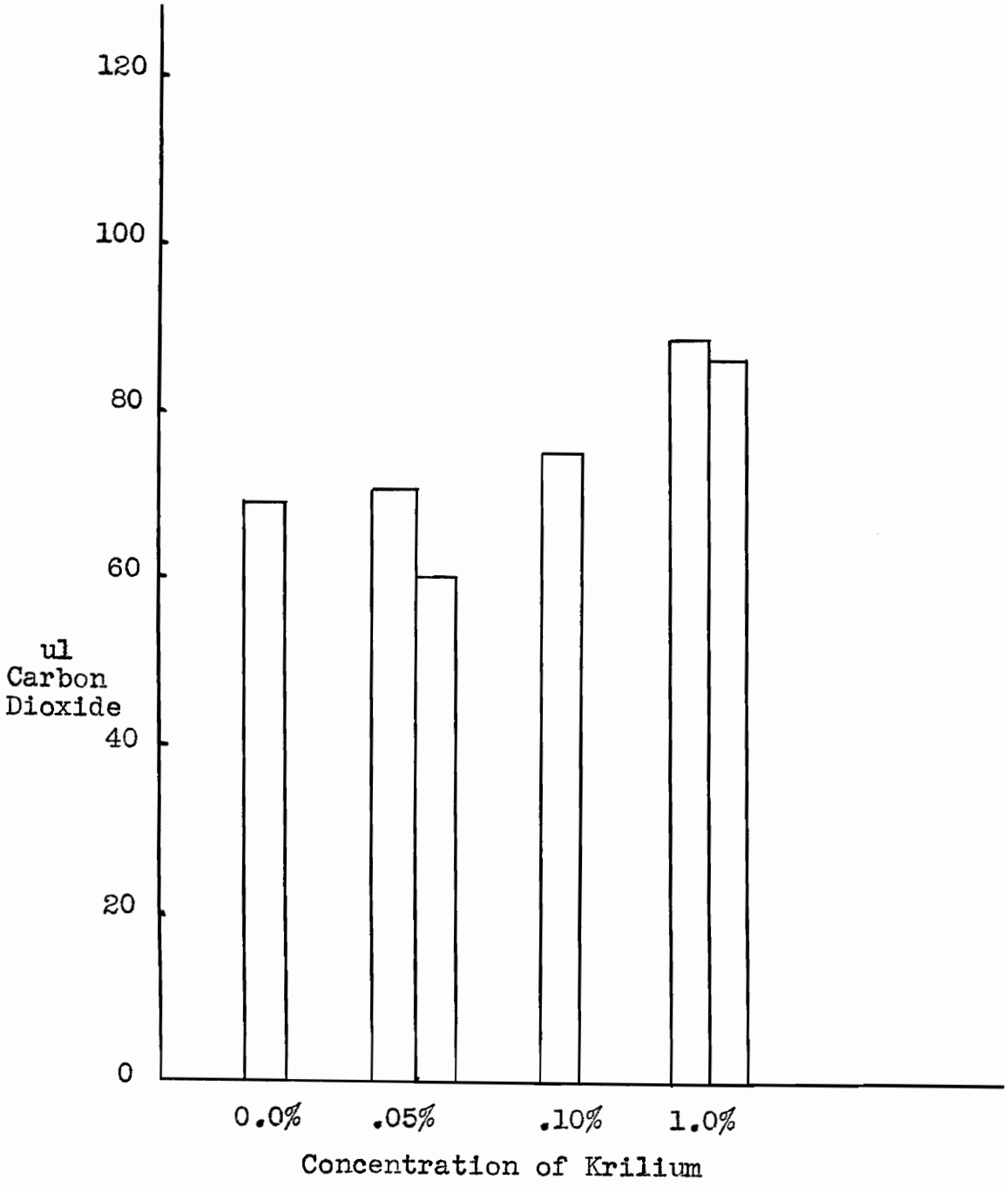

Figure 20. Carbon dioxide liberated by Agrobacterium tumefaciens on .02 M Glucose in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

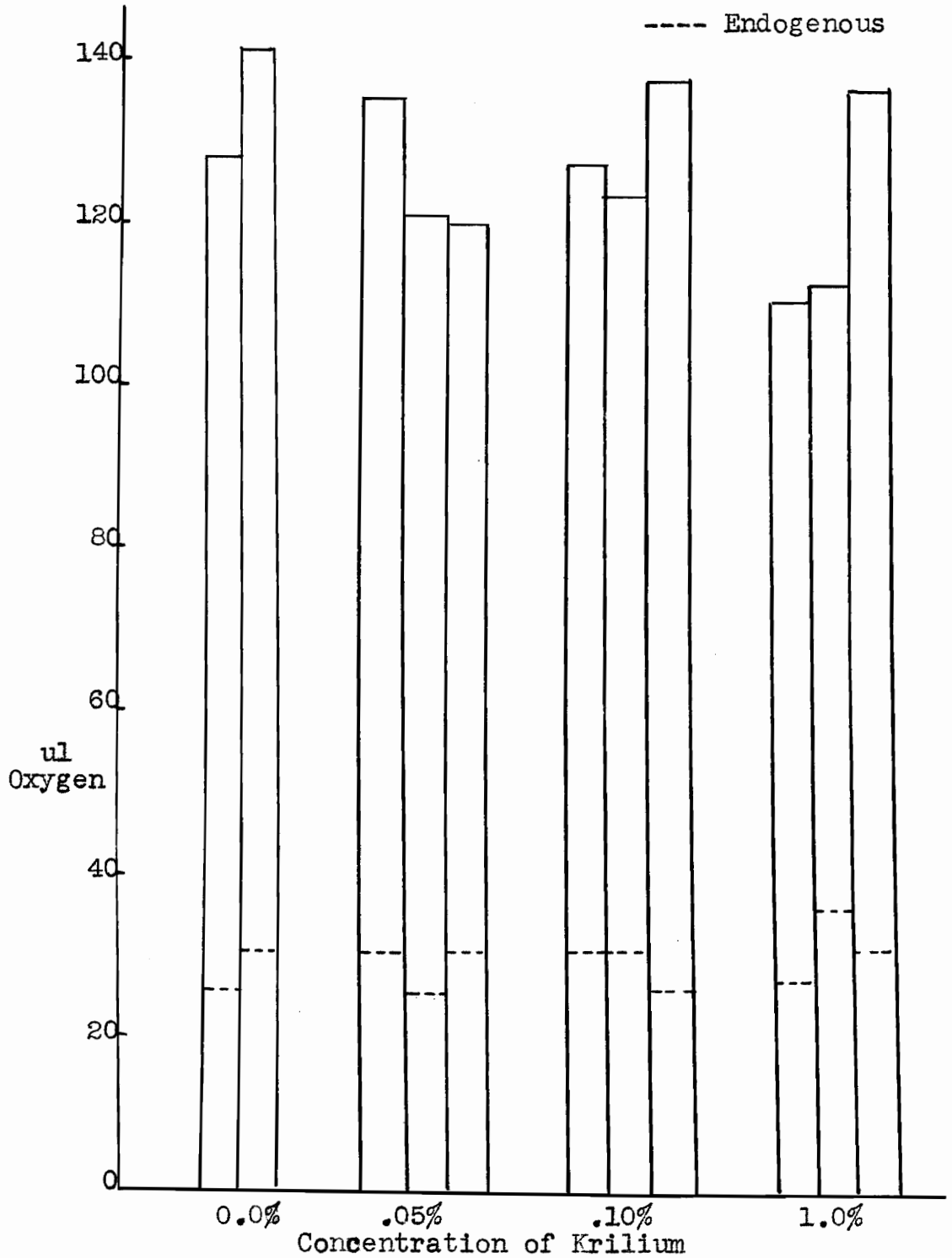

Figure 21. Oxygen uptake of Agrobacterium tumefaciens on .02 M Succinate in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

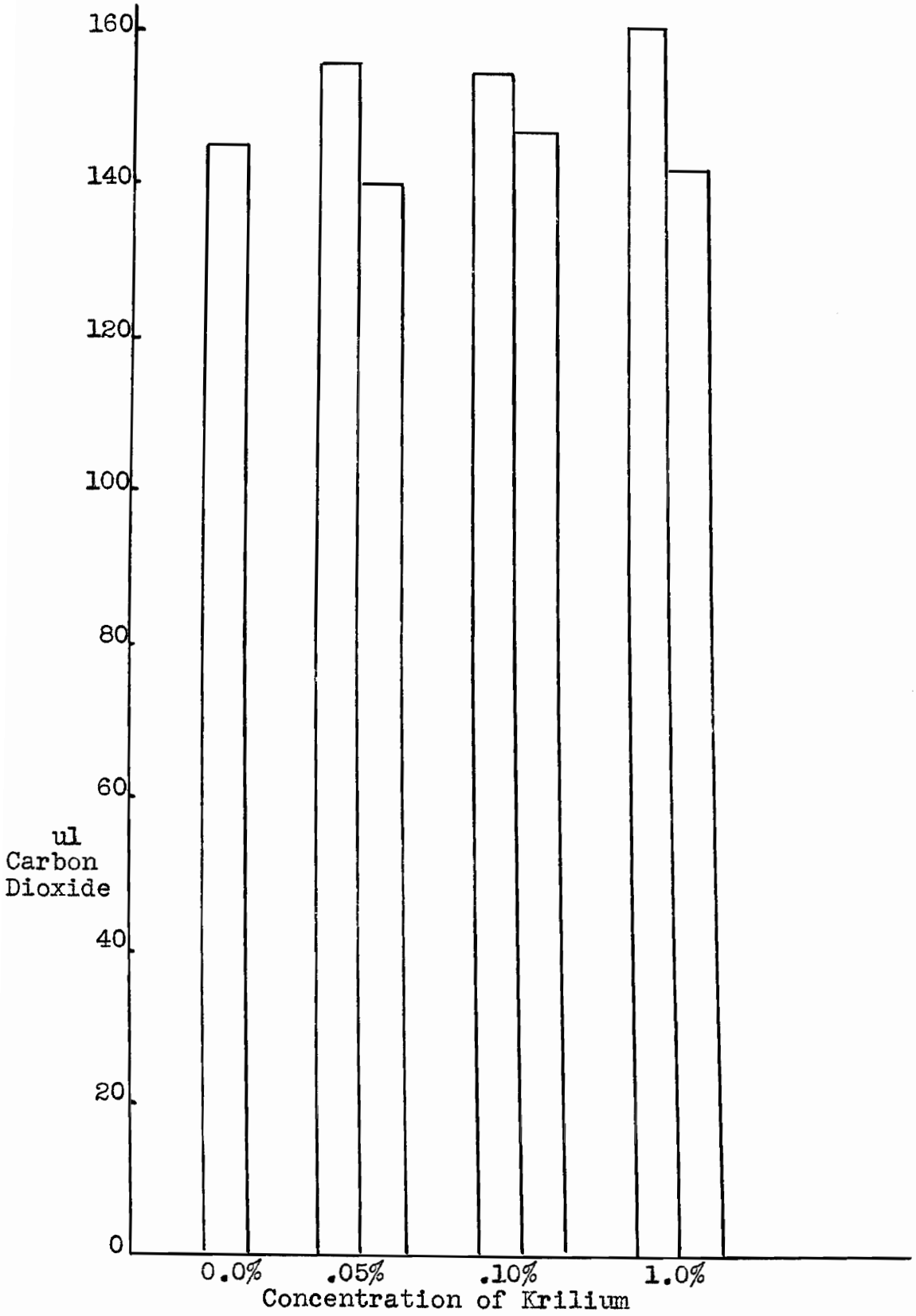

Figure 22. Carbon dioxide liberated by Agrobacterium tumefaciens on .02 M Succinate in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

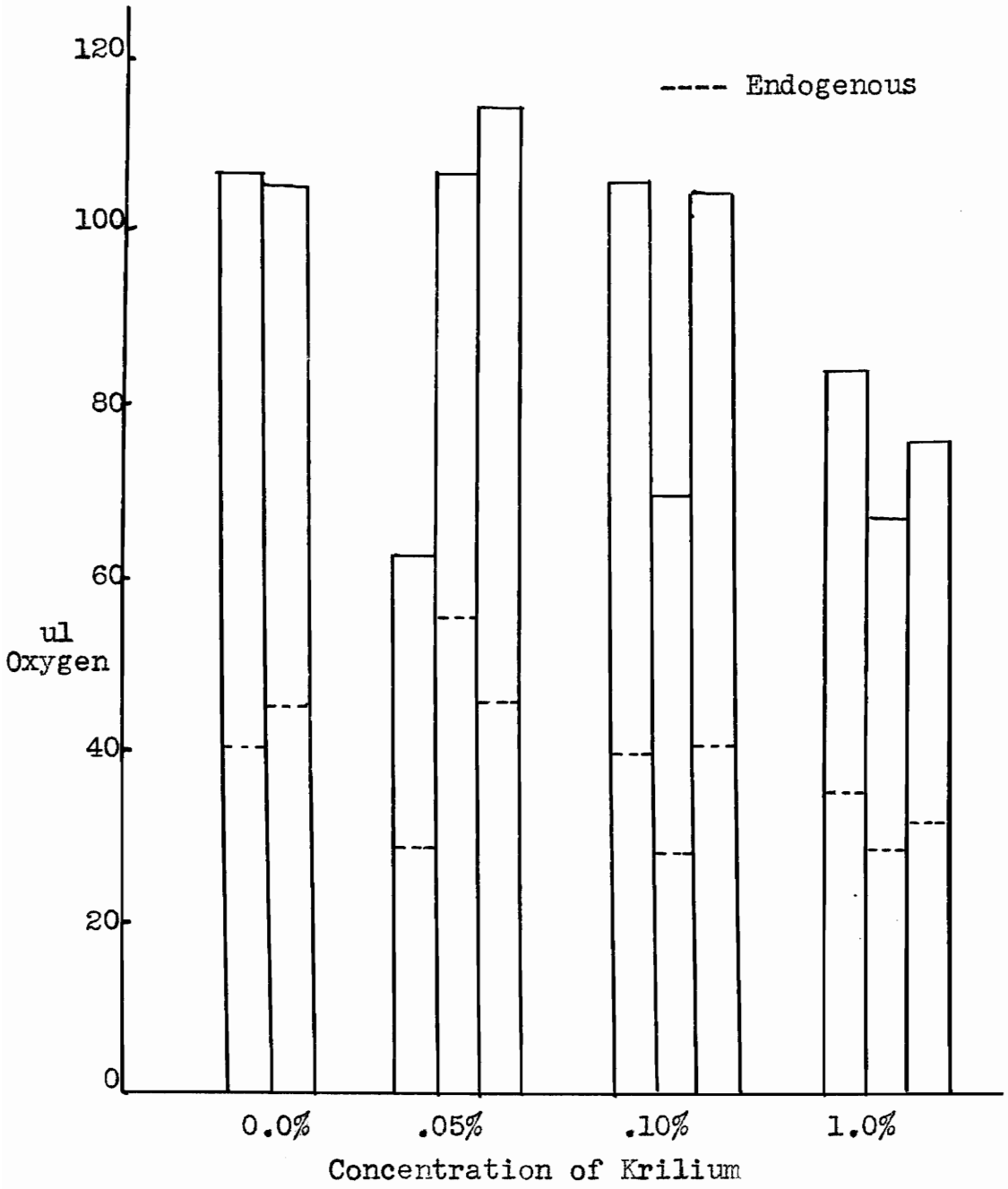

Figure 23. Oxygen uptake of Agrobacterium tumefaciens on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

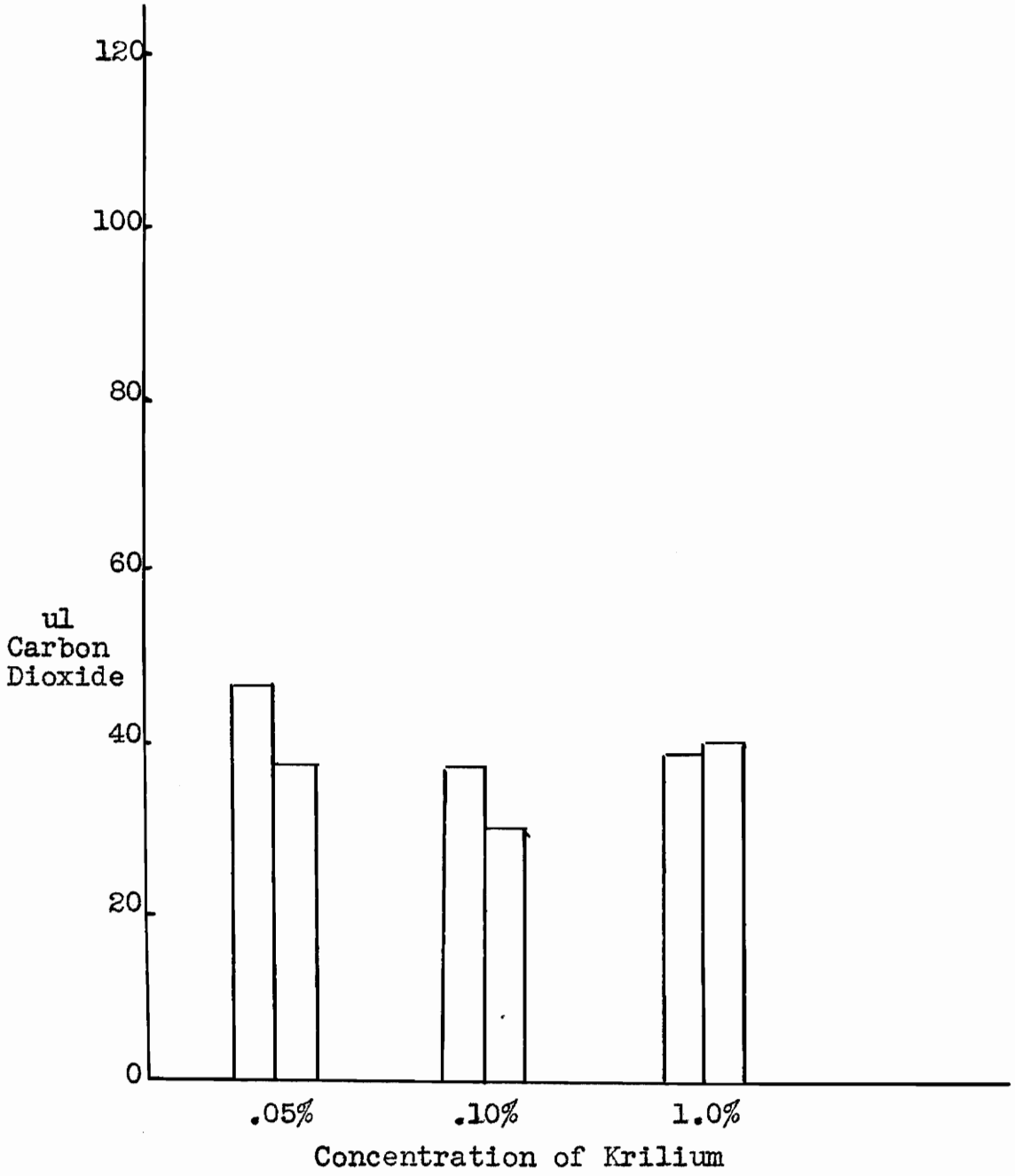

Figure 24. Carbon dioxide liberated by Agrobacterium tumefaciens on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

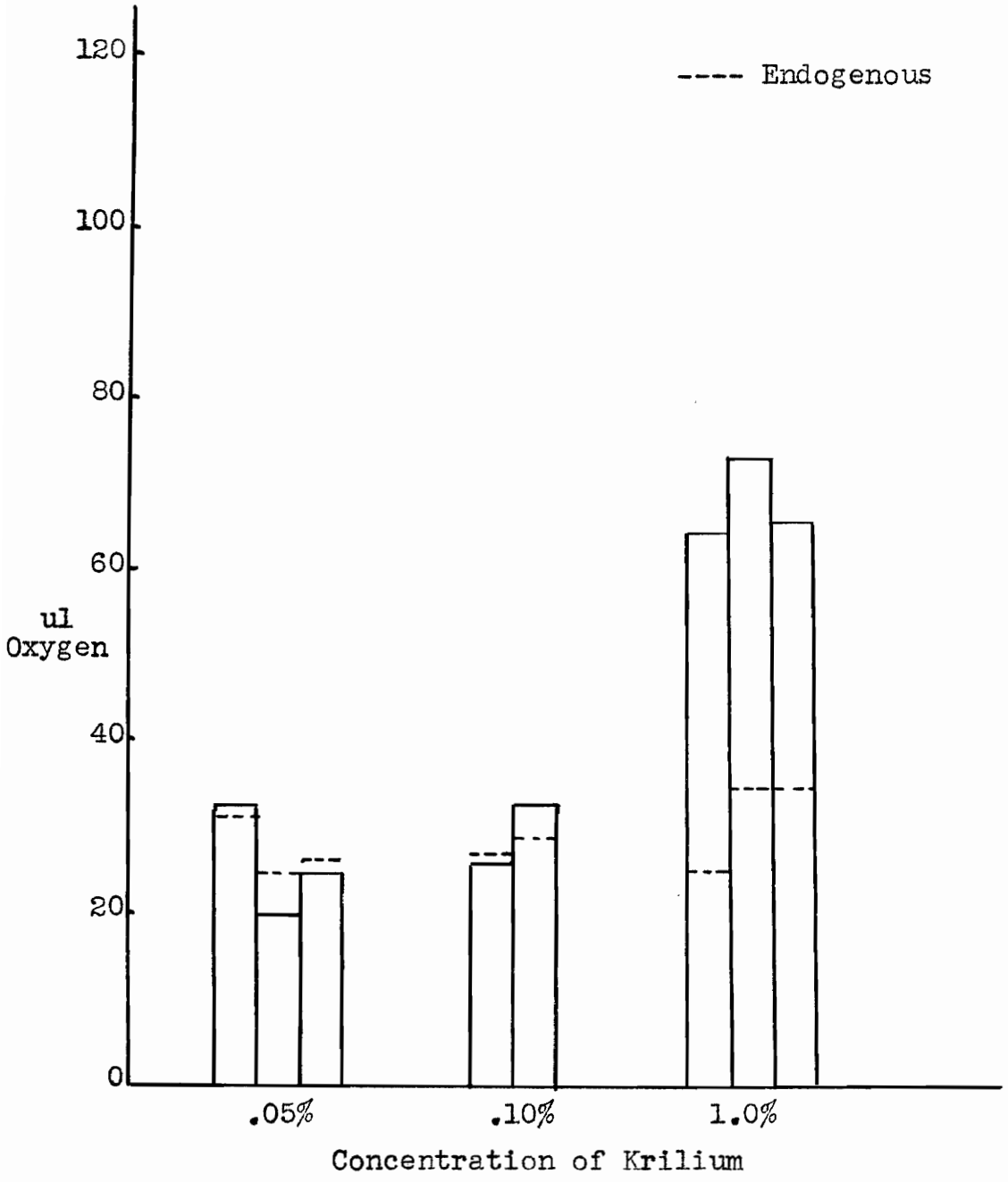

Figure 25. Oxygen uptake of Agrobacterium tumefaciens on shown concentrations of Krilium (Blend #6). 1.5 mg. cells Time is 90 minutes.

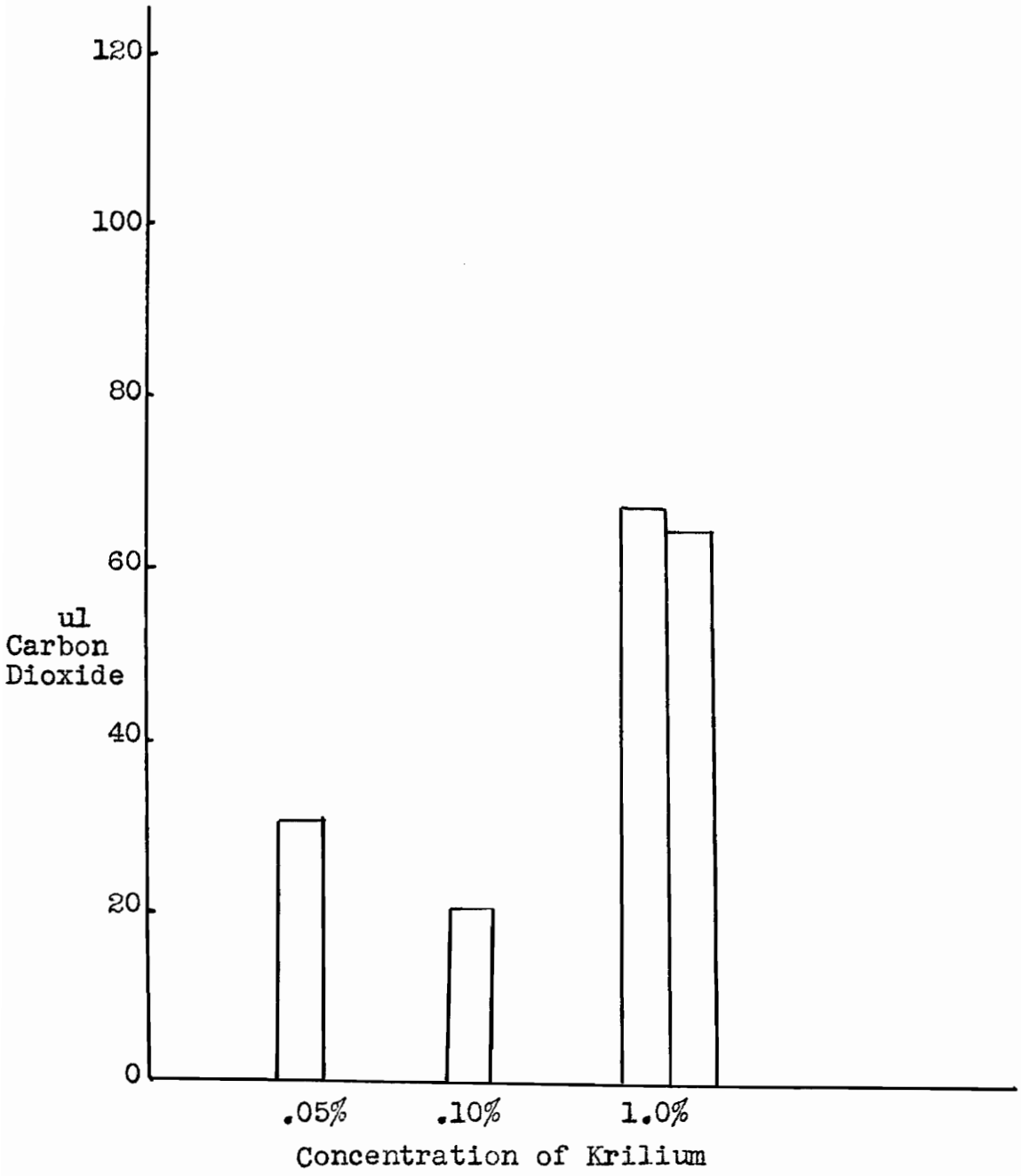

Figure 26. Carbon dioxide liberated by Agrobacterium tumefaciens on shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

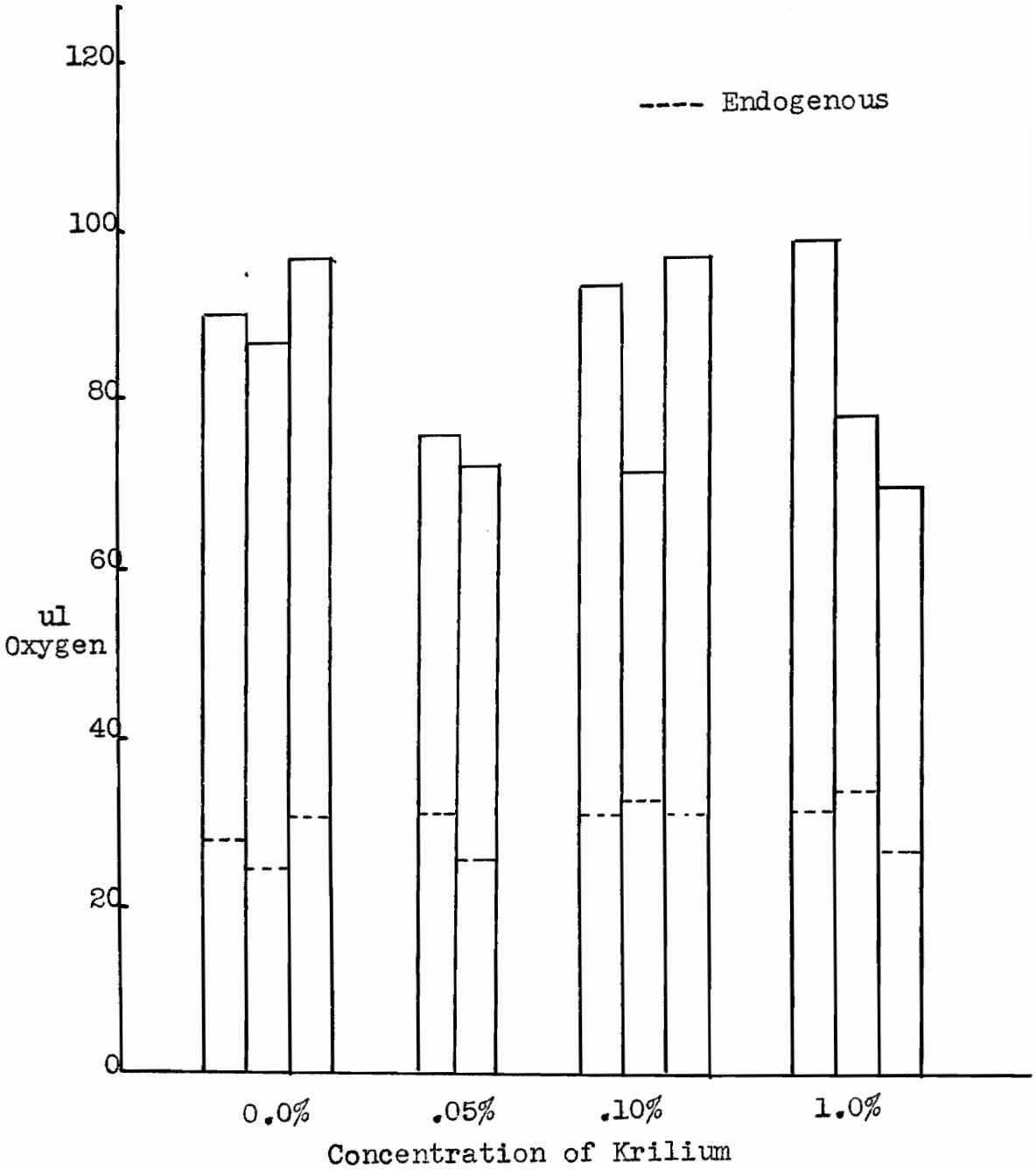

Figure 27. Oxygen uptake of Agrobacterium tumefaciens on .02 M Glucose in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

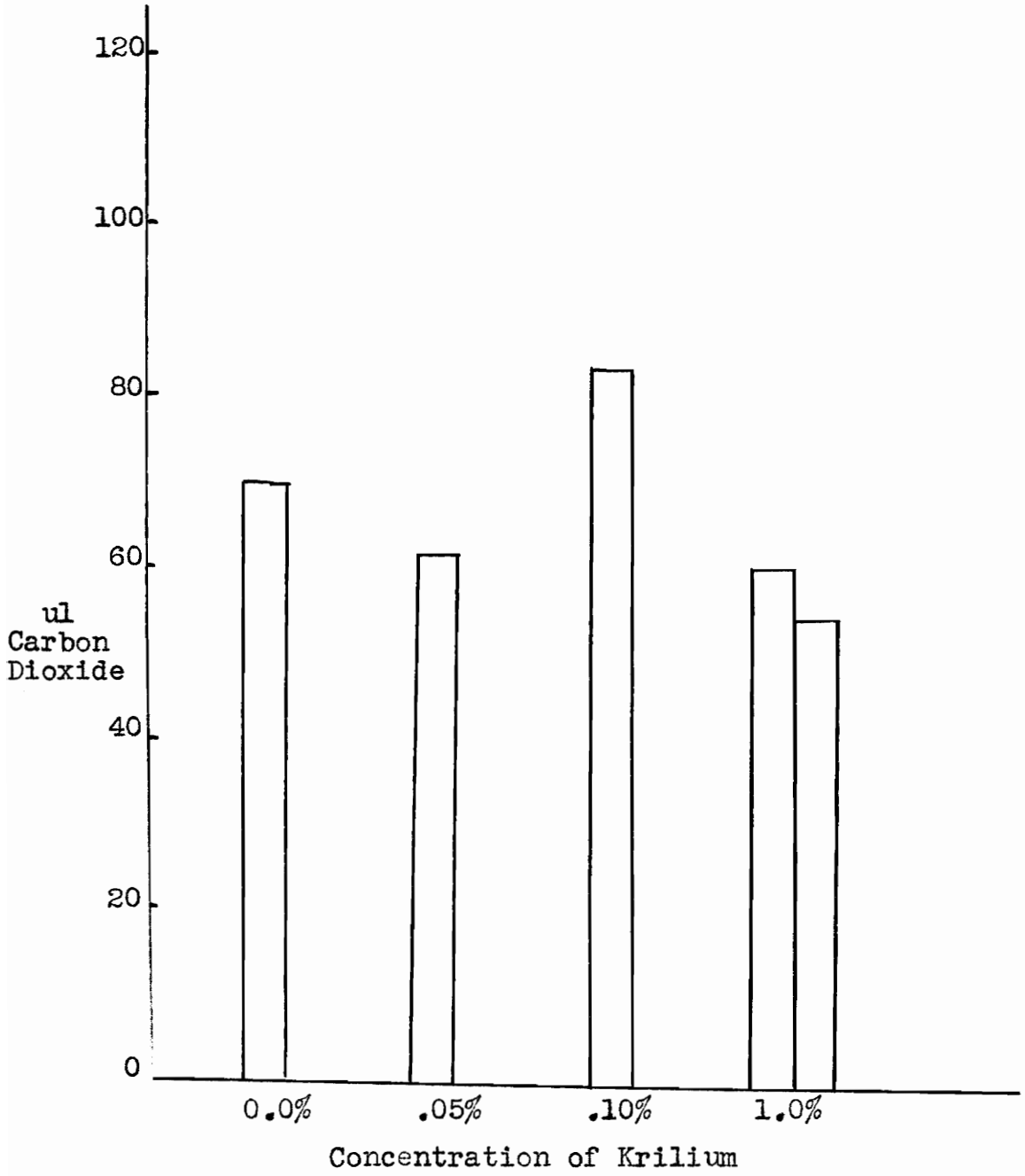

Figure 28. Carbon dioxide liberated by Agrobacterium tumefaciens on .02 M Glucose in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

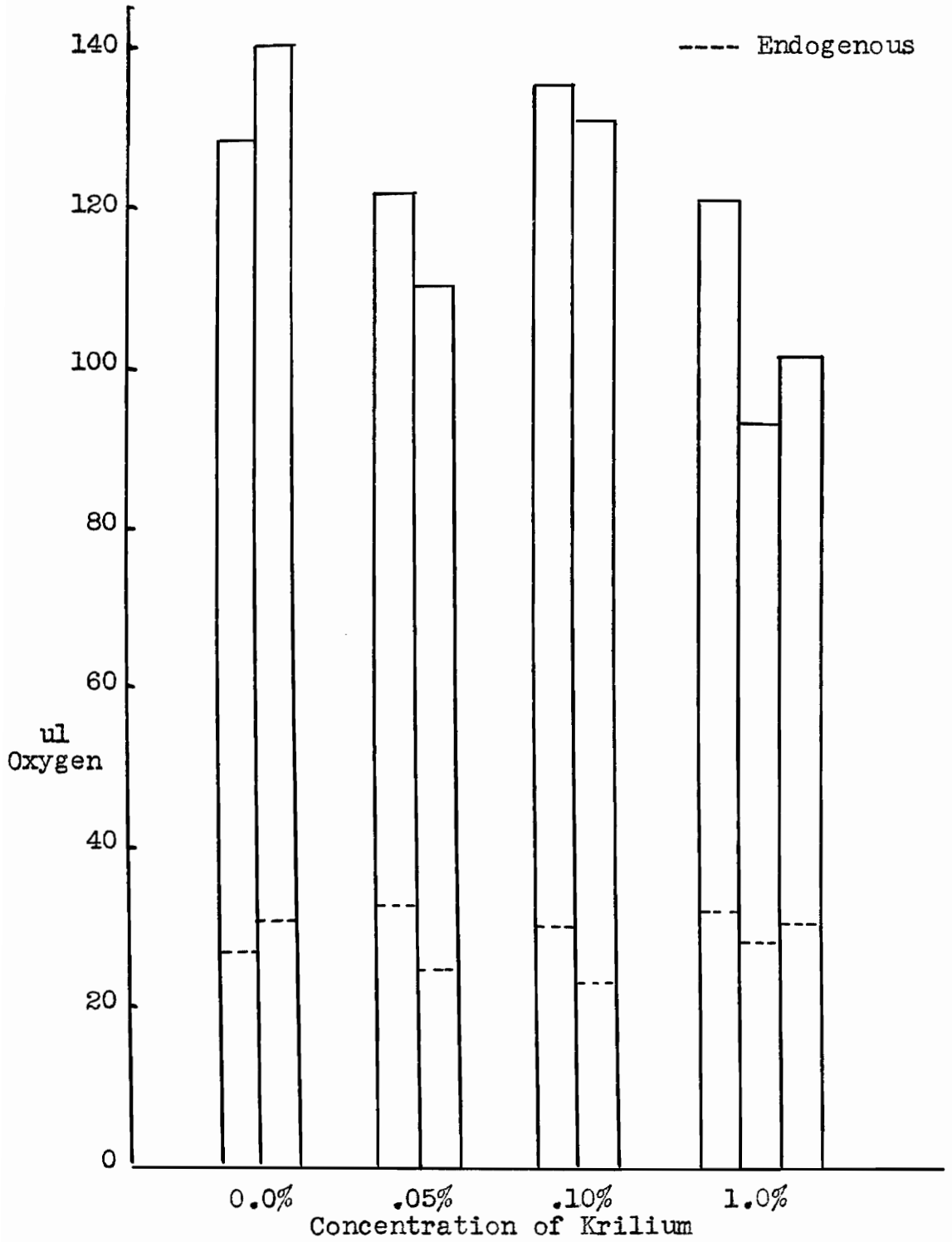

Figure 29. Oxygen uptake of Agrobacterium tumefaciens on .02 M Succinate in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

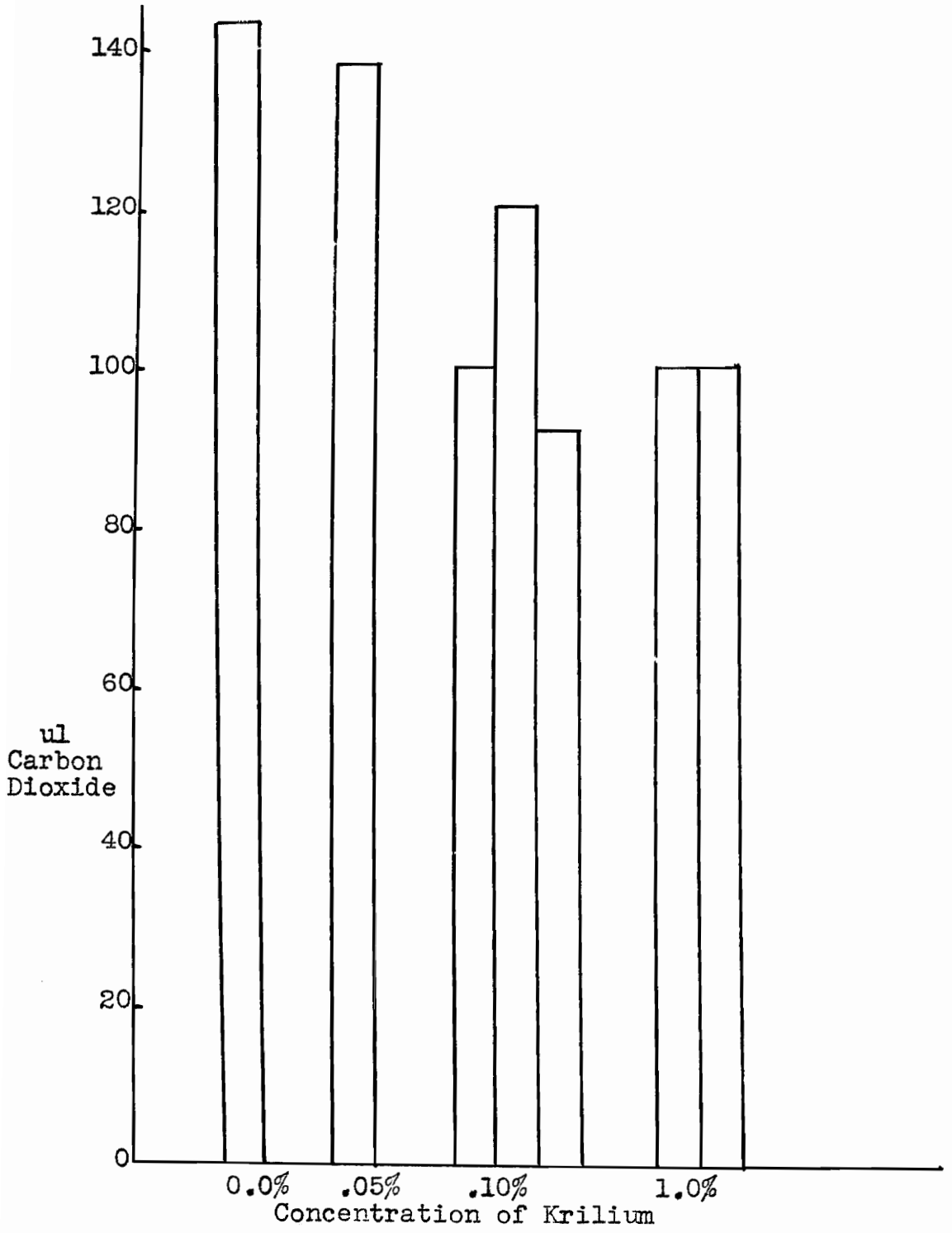

Figure 30. Carbon dioxide liberated by Agrobacterium tumefaciens on .02 M Succinate in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

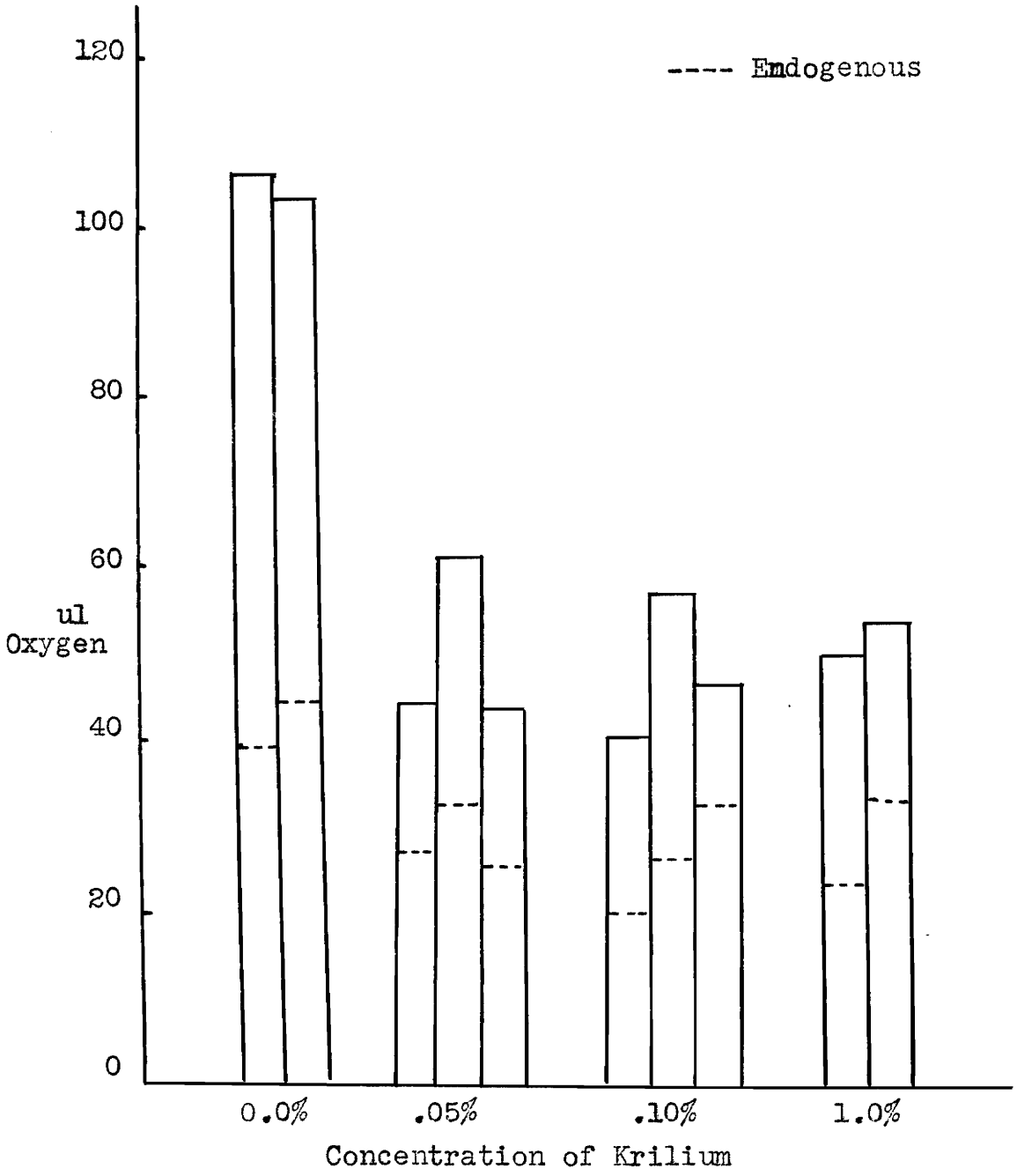

Figure 31. Oxygen uptake of Agrobacterium tumefaciens on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

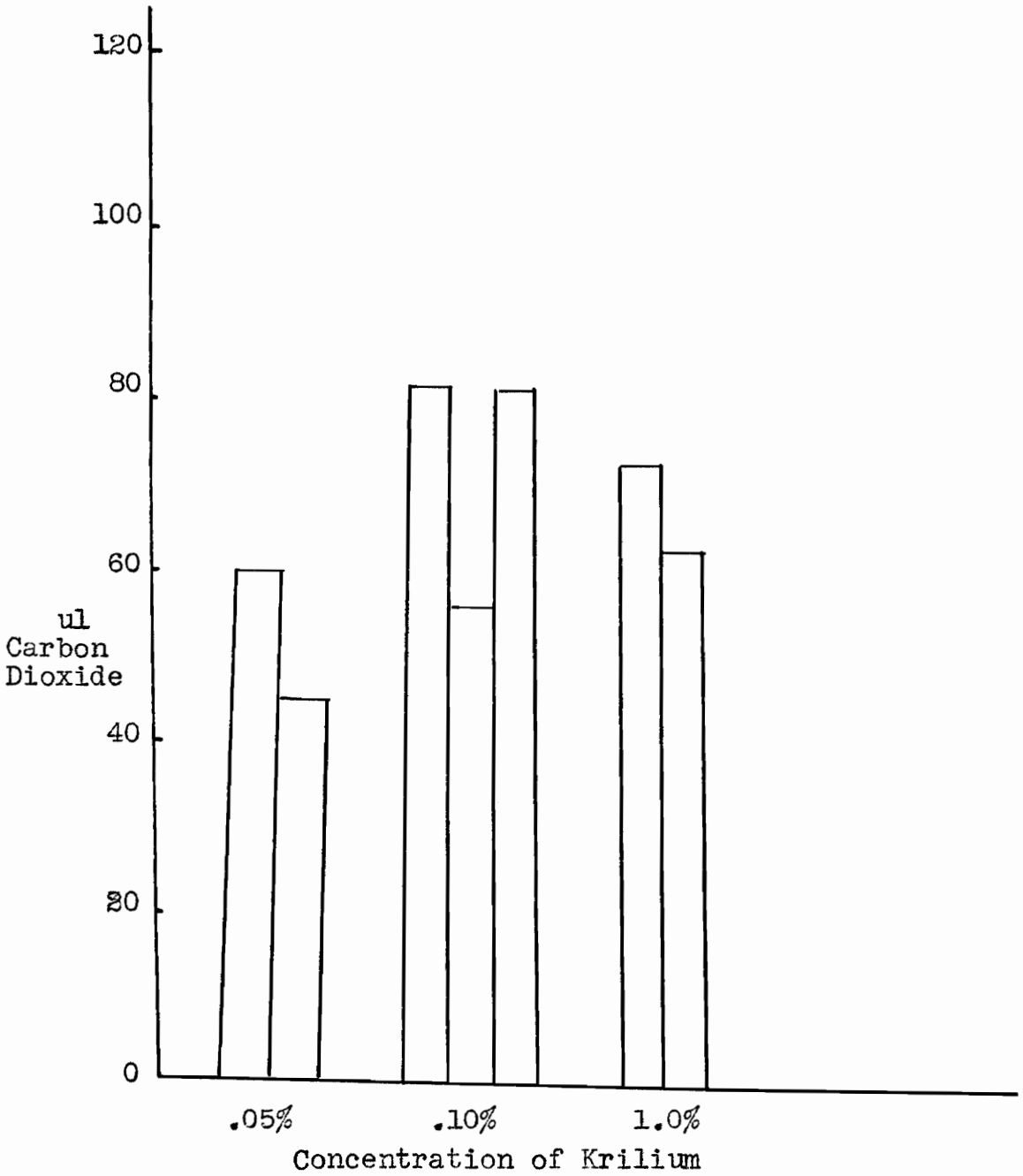

Figure 32. Carbon dioxide liberated by Agrobacterium tumefaciens on .02 M Mannitol in presence of shown concentrations of Krilium (Blend #6). 1.5 mg. cells - Time is 90 minutes.

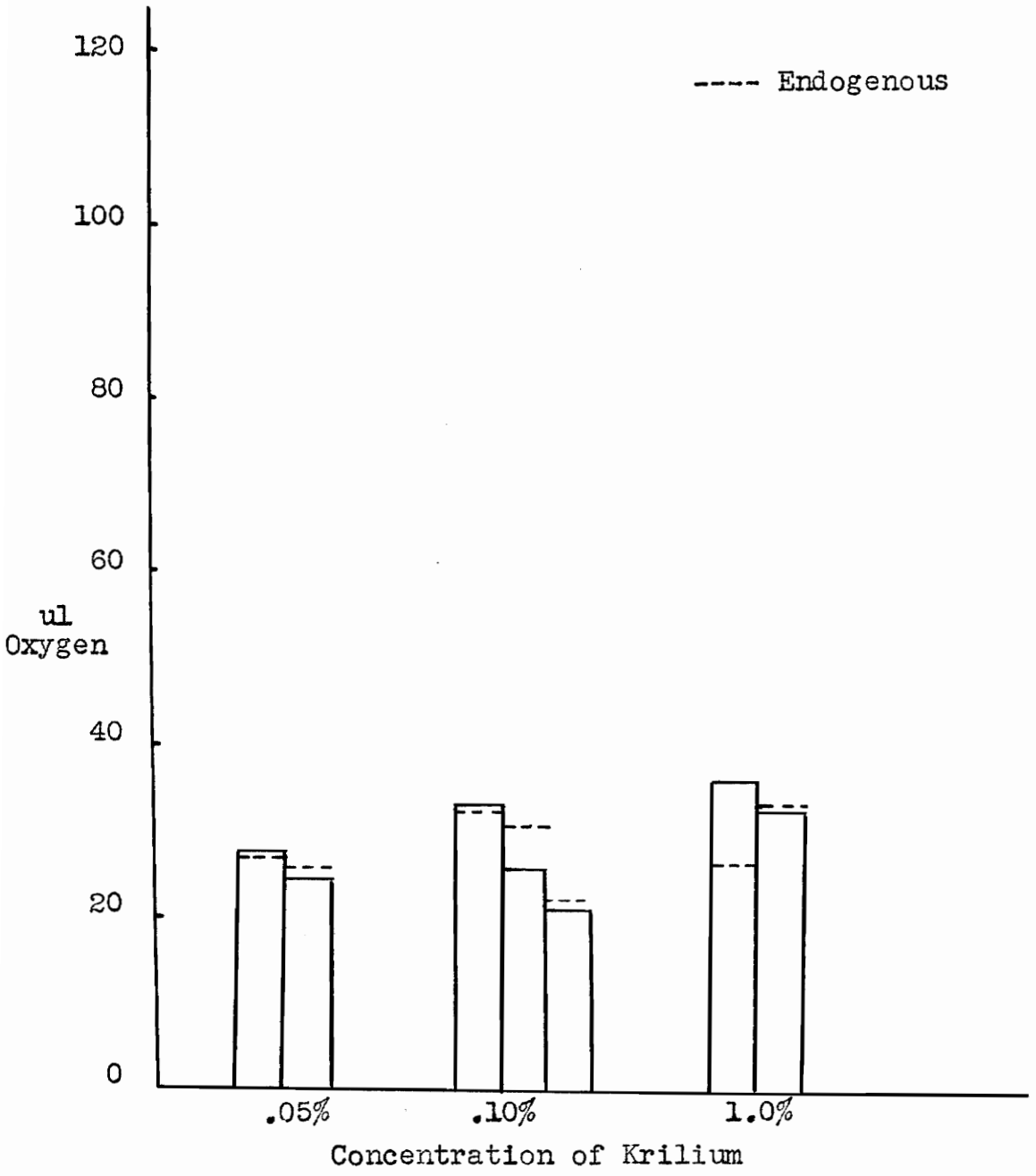

Figure 33. Oxygen uptake of Agrobacterium tumefaciens on shown concentrations of Krilium (Blend #9). 1.5 mg. cells Time is 90 minutes.

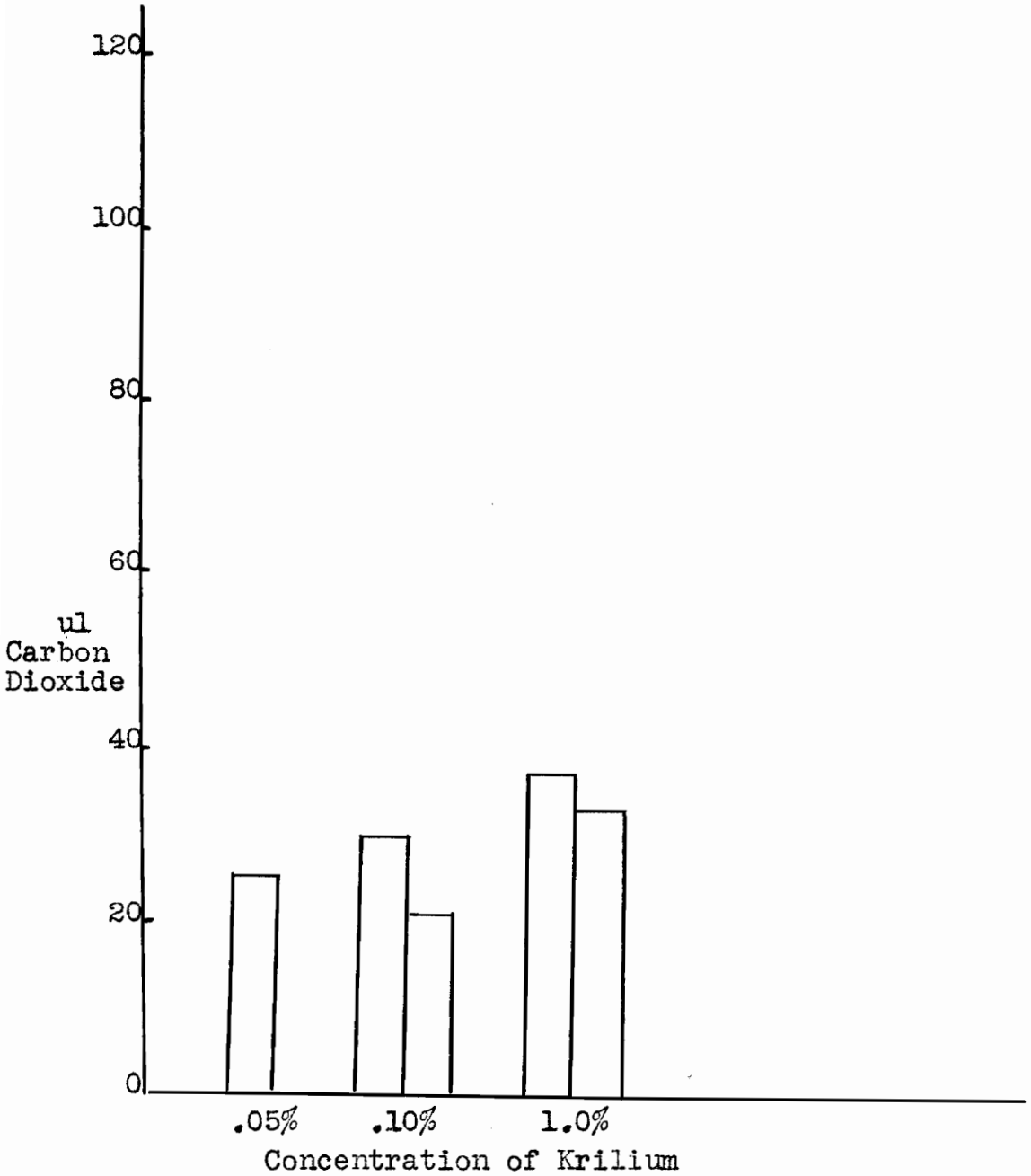

Figure 34. Carbon dioxide liberated by Agrobacterium tumefaciens on shown concentrations of Krilium (Blend #9). 1.5 mg. cells - Time is 90 minutes.

DISCUSSION OF RESULTS

The effect of Krilium on the respiratory activities of Rhizobium trifolii 205 and Agrobacterium tumefaciens was investigated by means of the Warburg respirometer. It was necessary to calibrate the Warburg apparatus and develope a technique for using this instrument before the investigation could be initiated. After the preliminary work was conducted, respiration measurements were made on the test organisms when these organisms were subjected to glucose, succinic acid and mannitol respectively. The oxygen uptake and carbon dioxide liberation was measured when each organism was introduced to each of the above substrates to which a given concentration (.05%m .10% and 1.0%) of Krilium had been added. Two blends of Krilium were tested in this investigation. These blends of Krilium were signified by Monsanto Chemical Company as Krilium (Blend #6) and Krilium (Blend #9).

The discussion on the respiratory activities of the two organisms, Rhizobium trifolii 205 and Agrobacterium tumefaciens, will be presented separately. Also the discussion of results pertaining to each specific substrate will be presented separately, including the results of oxygen uptake and carbon dioxide liberation of the organisms when subjected to the substrates containing either of the two blends of Krilium. A general discussion will be given in an attempt to explain the results obtained when Krilium was added to the

specific substrates.

Respiratory Activities of Rhizobium trifolii -

Respiration on Glucose - Krilium (Blend #6) - The respiratory rates of Rhizobium trifolii were determined when glucose was used as the substrate. These determinations were made to establish a reference point whereby comparisons could be made of results obtained from experiments in which .05%, .10% and 1.0% concentrations of Krilium had been added to the substrate, glucose. Replicate determinations were made and the results obtained were consistant.

It appears that the respiratory rate is slightly increased when Krilium is added to the substrate. The increase in concentration of Krilium from .05% to 1.0% seemed to have no significant effect on the rate obtained after the initial addition of .05% Krilium to the glucose. Approximately constant respiratory rates were obtained when the organism was subjected to the substrate containing the concentrations of Krilium. The results can best be seen by observing Figure 35, which shows the average oxygen uptake in presence of the varying concentrations of Krilium and substrate.

Krilium (Blend #9) - Determinations of the respiratory activities of the given organism were made using Krilium (Blend #9) in the same manner as in the case of Krilium (Blend #6). From the results obtained, it appears that the respiratory rate of Rhizobium trifolii is decreased slightly

when Krilium (Blend #9) is added to the glucose substrate in varying concentrations.

Respiration on Succinate - Krilium (Blend #6) - Gaseous exchange is increased to some extent when Krilium (Blend #6) is added to a substrate of succinic acid. There is a very slight increase in the oxygen uptake when the concentration of Krilium is increased. When the concentration of Krilium is increased, the amount of carbon dioxide liberated increases to a greater extent than does the oxygen uptake.

Krilium (Blend #9) - Oxygen uptake decreased consistantly as the concentration of Krilium is increased. Even though the oxygen uptake did decrease, the amount of carbon dioxide evolved increased directly with increasing concentrations of Krilium. It may be noted that the amount of carbon dioxide liberated is much greater when the 1.0% concentration of Krilium is used than with the other two concentrations.

Respiration on Mannitol - Krilium (Blend #6) - Strange respiratory rates were obtained for Rhizobium trifolii when mannitol containing Krilium was used as the substrate. When mannitol containing .05% Krilium was used, both the amount of oxygen absorbed and carbon dioxide evolved were reduced. The reduction in the respiratory rate was even greater when the concentration of Krilium was increased to .10%. The peculiar results appeared when the concentration of Krilium was increased to 1.0%. Instead of obtaining a decrease in the res-

piratory rate as was anticipated, results were obtained comparable to those obtained when mannitol containing no Krilium was used as the substrate.

Krilium (Blend #9) - Oxygen consumption was shown to be slightly decreased when the Krilium (Blend #9) was added to the mannitol substrate. A comparatively greater decrease in carbon dioxide liberation was demonstrated to accompany the decrease in oxygen consumption.

Respiration on Krilium - Measurements of the rate of gaseous exchange were made when the blends of Krilium were introduced as the substrates for the organism. The respiratory rates of the organism were observed on .05%, .10% and 1.0% solutions of Krilium.

Krilium (Blend #6) - The rate of respiration of Rhizobium trifolii on a .05% solution of Krilium was approximately the same as the endogenous respiration. When the concentration of Krilium was increased to .10%, the rate of respiration increased and continued to increase to a greater extent when a 1.0% solution of Krilium was used as the substrate.

Krilium (Blend #9) - Respiration of Rhizobium trifolii on Krilium (Blend #9) was very nearly the same as the endogenous respiration. In some cases the rate was even less than the endogenous rates obtained, but on an average, the rates were the same as the endogenous respiration.

General Discussion - The effect Krilium (Blend #6) had on the respiratory rates of Rhizobium trifolii was not the same for each substrate. In the case where glucose, succinic acid and Krilium (Blend #6) were used as the substrates, an increase in respiratory rates was obtained. When mannitol was the substrate in the presence of .05% and .10% Krilium, there was a considerable decrease in the respiratory rate. Increasing the concentration of Krilium to 1.0% brought about a respiratory rate similar to that obtained when no Krilium was present.

A decrease in respiration was noticeable in each case when Krilium (Blend #9) was added to the substrate.

Respiratory Activities of Agrobacterium tumefaciens -

Respiration on Glucose - Krilium (Blend #6) - The respiratory rates of Agrobacterium tumefaciens were effected to some extent when a .05% and .10% concentration of Krilium was added to the glucose substrate. The .05% solution deminished the respiratory rate to a greater extent than did the .10% solution of Krilium. In the presence of a 1.0% solution of Krilium, the activities of the organisms were the same as those obtained when no Krilium had been added to the substrate.

Krilium (Blend #9) - A very noticeable decrease in respiratory rates was observed upon the addition of a .05% solution of Krilium to the substrate. After an increase in con-

centration to .10% Krilium, the respiratory activities approached the control but was still lower than when no Krilium was present in the substrate. Similar results were obtained when the concentration of Krilium was increased to 1.0%.

Respiration on Succinate - Krilium (Blend #6) - A consistant decrease in oxygen consumption by Agrobacterium tumefaciens was observed when Krilium was added in increased concentrations to the succinate substrate.

Krilium (Blend #9) - A more significant decrease in respiratory rates was observed when Krilium (Blend #9) was used than when Krilium (Blend #6) was added to the substrate. A 17% decrease in total oxygen consumption was noted when the concentration of Krilium was increased to a 1.0% level.

Respiration on Mannitol - Krilium (Blend #6) - No significant change in respiratory activities was observed when the .05% and .10% solutions of Krilium were placed in the mannitol substrates. Yet, when the concentration of Krilium was increased to a 1.0% solution, the oxygen consumption showed a 25% decrease.

Krilium (Blend #9) - The activities of Agrobacterium tumefaciens were greatly inhibited when Krilium (Blend #9) was added to the mannitol substrate. In the presence of .05%,.10% and 1.0% solutions of Krilium, the oxygen con-

sumption of this organism was deminished to approximately one-half of its oxygen consumption when subjected to a mannitol substrate containing no Krilium (Blend #9).

Respiration on Krilium - Krilium (Blend #6) - As demonstrated in Figure 42, the oxygen consumption of Agrobacterium tumefaciens increased considerably when subjected to a substrate containing only Krilium.

Krilium (Blend #9) - There was a noticeable increase in oxygen consumption when the organism was permitted to respire on Krilium (Blend #9) as the substrate. The increase in the respiratory rate was not as great as that obtained for Krilium (Blend #6).

General Discussion - Results obtained, when Agrobacterium tumefaciens was permitted to respire on the substrates containing Krilium (Blend #6), seemed to indicate that there was a slight inhibitory effect of the Krilium on the respiratory rates of the organism as the concentration of Krilium was increased to a maximum of 1.0%. Addition of a .05% and .10% concentration of Krilium to the substrate seemed to have a very slight effect on the respiration of the organism.

Krilium (Blend #9) had a more adverse effect on the rate of respiration than did Krilium (Blend #6). A very noticeable decrease in oxygen consumption was observed when Kril-

ium (Blend #9) was added to the three substrates.

An unexplained observation was made when Krilium was used as the substrate. The oxygen consumption increased as the concentration of Krilium was increased. Although the Krilium seems to have a slight inhibitory effect on the respiration of the organism when added to some other substrate, there is an increase in oxygen consumption when Krilium is used as the substrate. This indicates that the organisms are able to utilize the Krilium to some extent.

The results obtained for both organisms can be better illustrated by Figures 35 - 42. The microliters of oxygen consumed by the organisms represents the average of the determinations made for the given conditions as stated in each of the following figures.

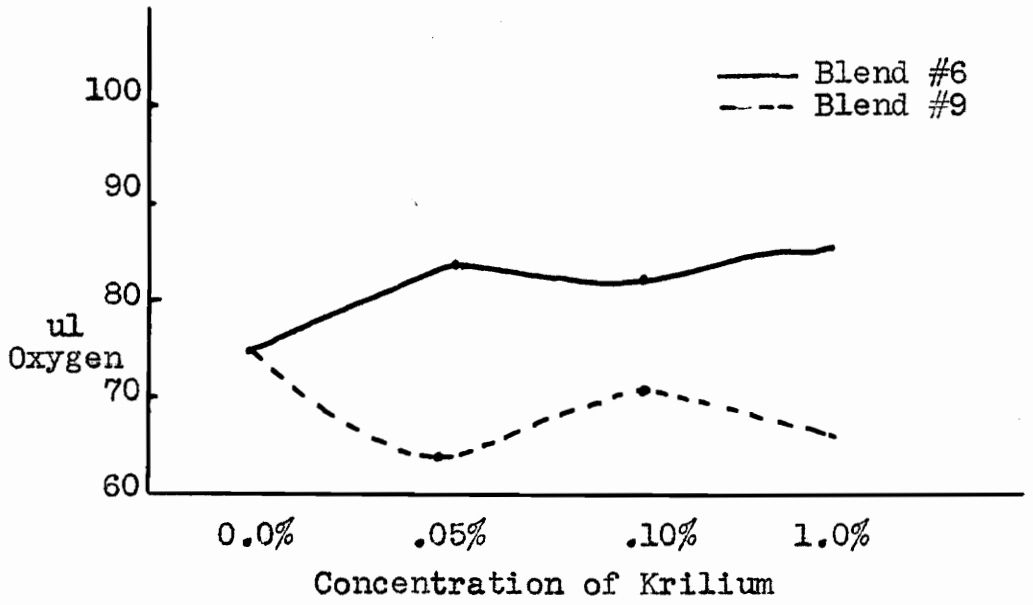

Figure 35. Oxygen uptake of Rhizobium trifolii on .02 M Glucose in presence of shown concentrations of Krilium.

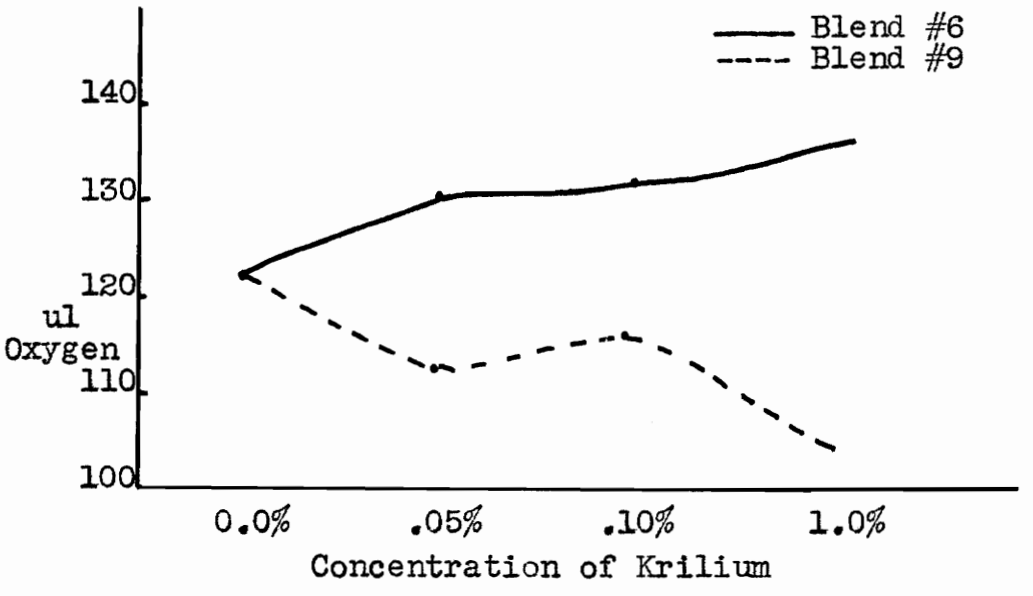

Figure 36. Oxygen uptake of Rhizobium trifolii on .02 M Succinate in presence of shown concentrations of Krilium.

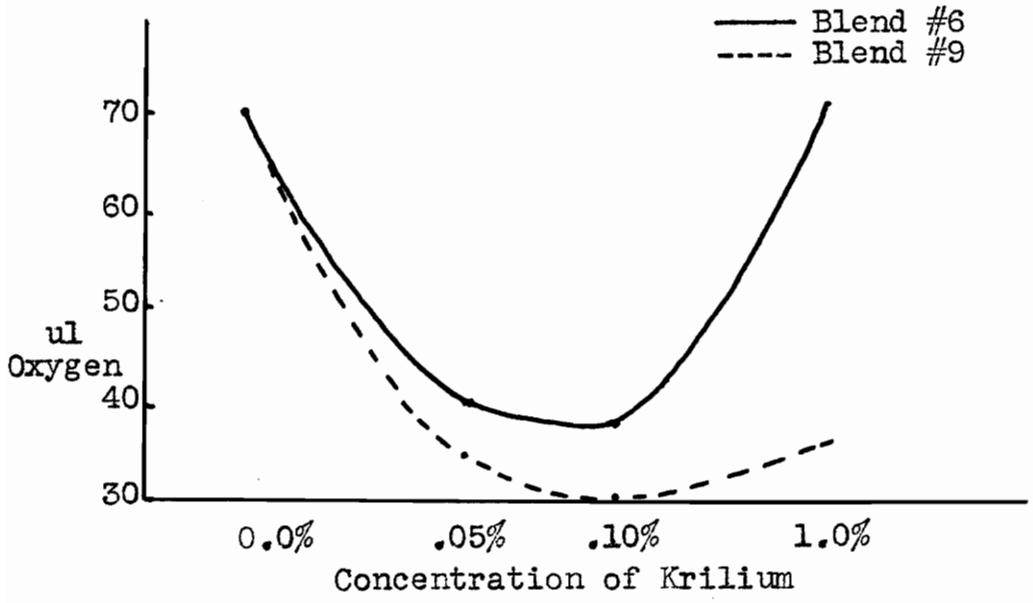

Figure 37. Oxygen uptake of Rhizobium trifolii on .02 M Mannitol in presence of shown concentrations of Krilium.

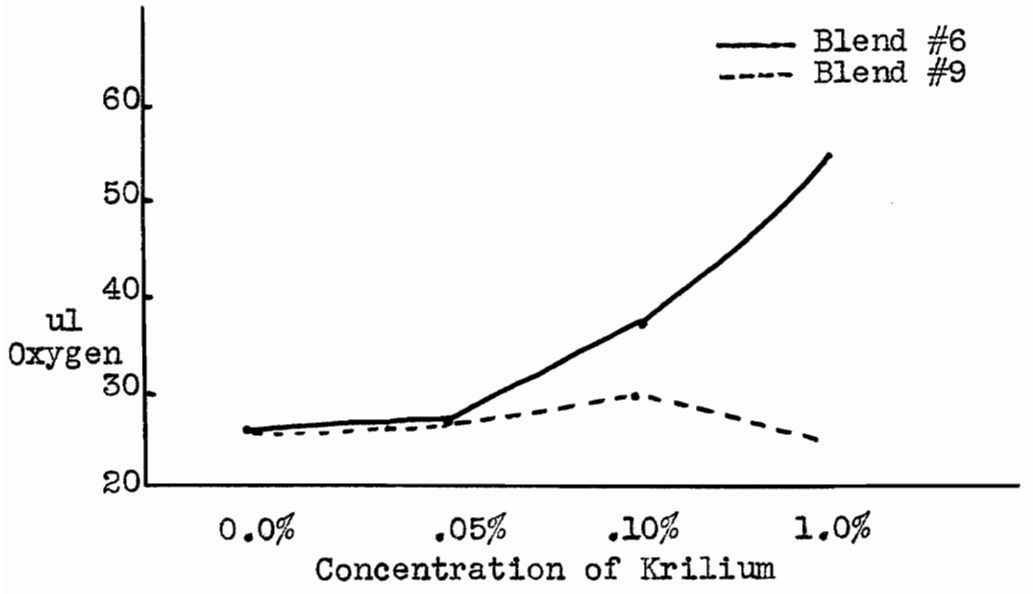

Figure 38. Oxygen uptake of Rhizobium trifolii on shown concentrations of Krilium.

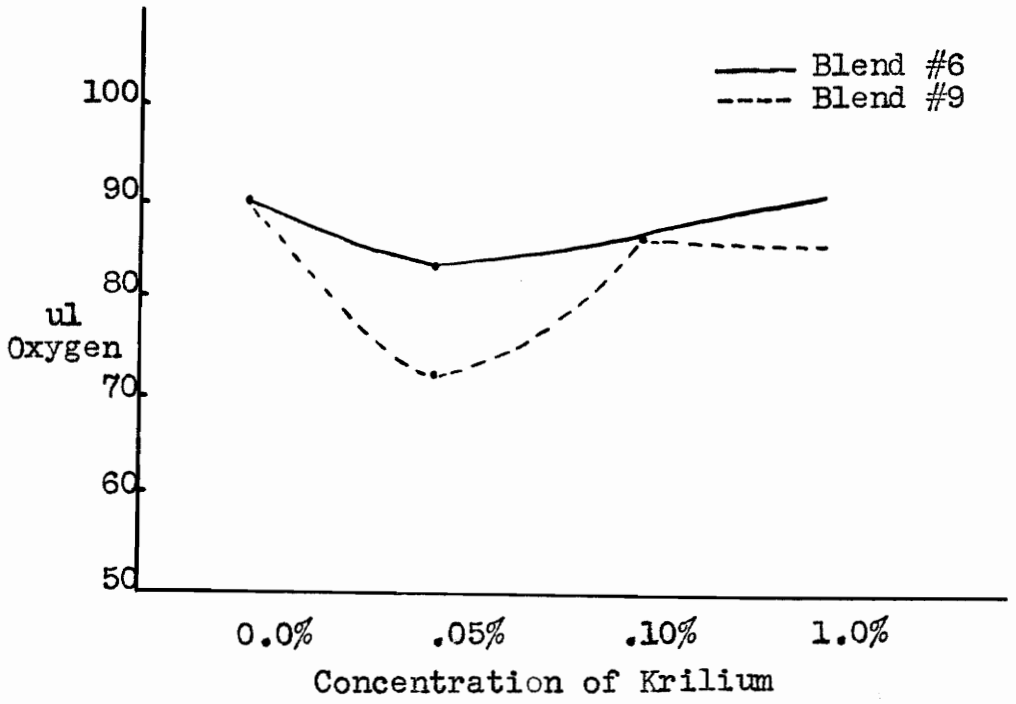

Figure 39. Oxygen uptake of Agrobacterium tumefaciens on .02 M Glucose in presence of shown concentrations of Krilium

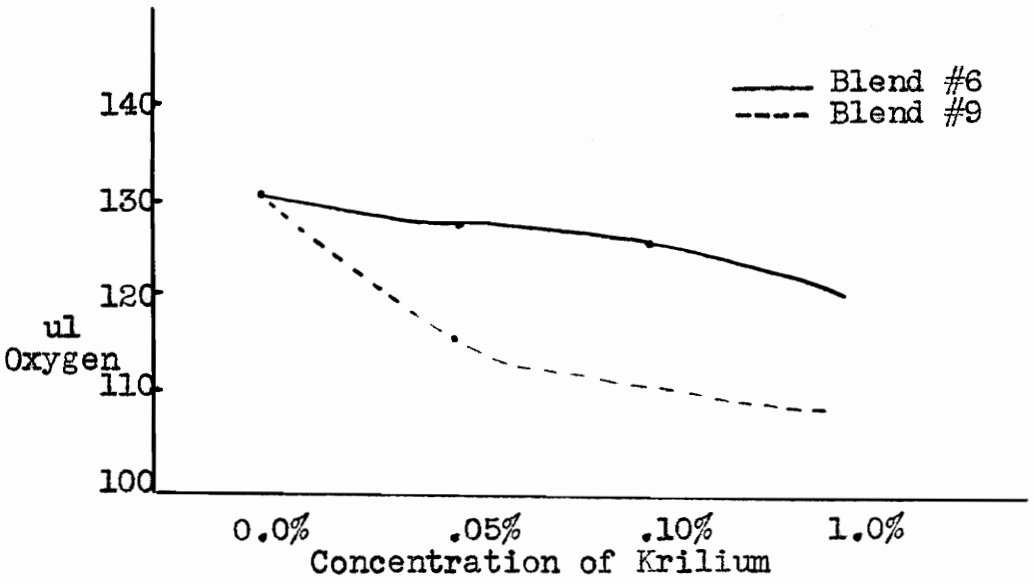

Figure 40. Oxygen uptake of Agrobacterium tumefaciens on .02 M Succinate in presence of shown concentrations of Krilium.

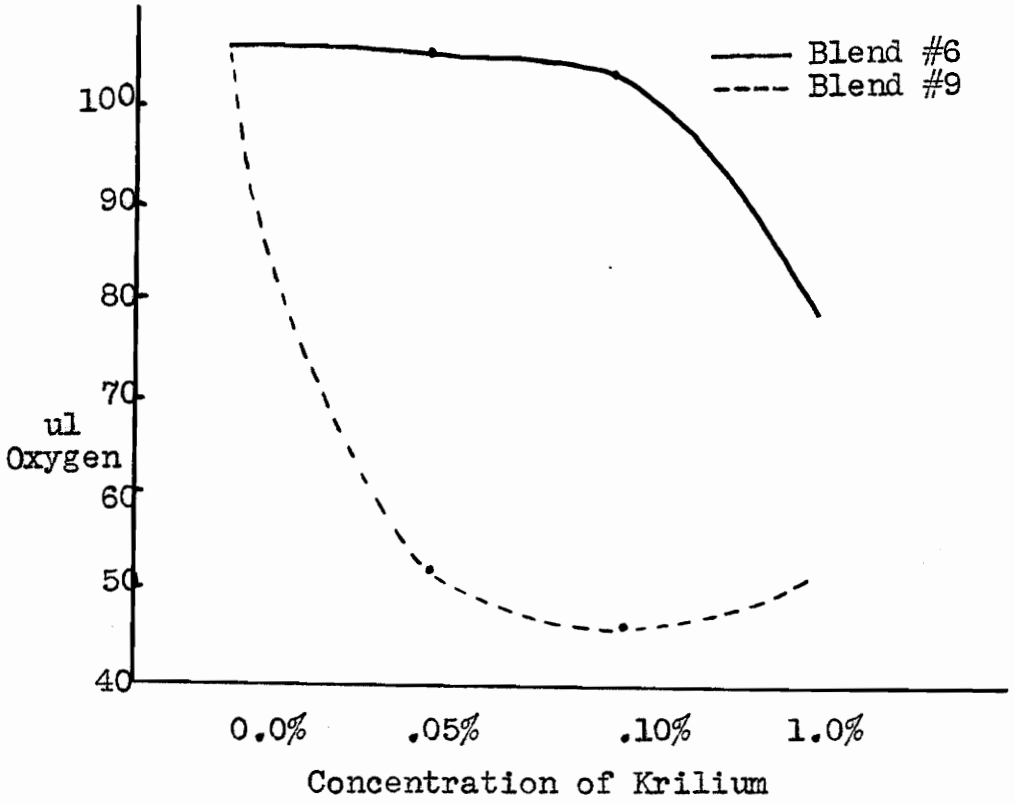

Figure 41. Oxygen uptake of Agrobacterium tumefaciens on .02 M Mannitol in presence of shown concentrations of Krilium.

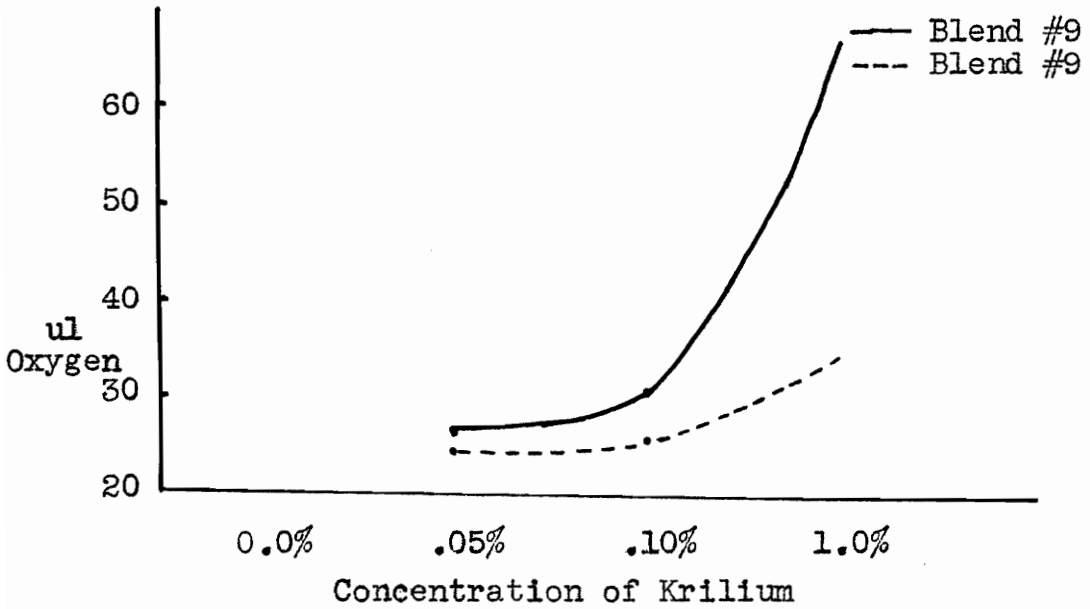

Figure 42. Oxygen uptake of Agrobacterium tumefaciens on shown concentrations of Krilium.

SUMMARY AND CONCLUSIONS

The respiratory rates of Rhizobium trifolii and Agrobacterium tumefaciens were measured by use of a Warburg Respirometer. A non-proliferating or "resting cell" technique was utilized in demonstrating the effect of Krilium on the oxygen consumption of the organisms when Krilium was added to a given substrate, glucose, succinate or mannitol.

Two types of Krilium were used in this investigation, Krilium (Blend #6) and Krilium (Blend #9). Observations were made on the effect of various concentrations (.05%, .10% and 1.0%) of the two blends of Krilium on the respiratory activities of the test organisms.

It was observed that Krilium (Blend #6) caused a noticeable increase in the respiratory rate of Rhizobium trifolii, whereas in the case of Agrobacterium tumefaciens, no effect or a slight decrease was observed when Krilium (Blend #9) was added to the substrate.

A very noticeable decrease in respiratory rates was observed when both test organisms were permitted to respire on substrates containing Krilium (Blend #9).

When Krilium (Blend #6) and Krilium (Blend #9) were used as the only substrates, an increase in respiration rates of the organisms was observed.

It is indicative from this work that Krilium (Blend #6)

either causes a slight increase in the respiratory activities or has no deleterious effect on the respiration of the test organisms. The results obtained, when using Krilium (Blend #9), seem to indicate that this blend of Krilium has an undesirable effect on the respiration of the two organisms used in this investigation.

REFERENCES

1. Allen, O. N., Experiments In Soil Bacteriology, Burgess Publishing Company, Minneapolis, Minn., 1950, page 66.

2. Baver, L., Soil Physics, John Wiley & Sons, Inc., New York, 1940, page 125 - 138.

3. Dixon, M., Manometric Methods as Applied to the Measurement of Cell Respiration and Other Processes, 2nd Ed., The MacMillan Company, 1943.

4. Georgi, C. E. and Wilson, P. W., The Influence of the Tension of Oxygen on the Respiration of Rhizobia, Archives Mikrobiology, Vol. 4, 1933, 543 - 564.

5. Gortner, R. A., Jr. and Gortner, W. A., Outlines of Biochemistry, John Wiley & Sons, Inc., New York, 3rd Edition, 82 - 83.

6. Hedrick, R. M. and Mowry, D. T., Effect of Synthetic Polyelectrolytes on Aggregation, Aeration and Water Relationships of Soil, Soil Science, Vol. 73, No. 6, June 1952, 427 - 441.

7. Krilium, Resin Soil Conditioner, Monsanto Chemical Company, St. Louis, Missouri.

8. Millar, C. E., Turk, L. M., Fundamentals of Soil Science, John Wiley & Sons, Inc., New York, 3rd Edition, 1951, 54 - 59.

9. Neal, O. R. and Walker, R. H., Physiological Studies

on Rhizobium. IV. The extent of oxidation of carbonaceous materials, Journal Bact., Vol. 32, 1936, 183 - 194.

10. Quastel, J. H., Influence of Organic Matter on Aeration and Structure of Soil, Soil Science, Vol. 73, June 1952, 419 - 426.

11. Ruehrwein and Ward, D. W., Mechanism of Clay Aggregation, Soil Science, Vol. 73, June 1952, 485 - 492.

12. Russel, E. W., Imperial Bureau of Soil Science Technical Communication No. 37, Soil Structure, 1938.

13. The Application of Monsanto Krilium for Improving Agricultural Soil Structure and Erosion Control, Development Data Report, Monsanto Chemical Company, St. Louis, Missouri.

14. Umbriet, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism for the Study of Tissue Metabolism, Burgess Publishing Company, Minneapolis, Minn., 1949.

15. Wilson, P. W., Respiratory Enzyme Systems in Symbiotic Nitrogen Fixation. I. The "resting cell" technique as a method for study of bacterial metabolism, Journal Bact. 35, 1938, 601 - 623.

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Spouse: Jo Ann Craft Stafford

Education: Burlington City Schools 1934 - 1945

Elon College A. B. Degree 1945 - 1949

Organizations: Phi Sigma Biological Society

Phi Lambda Upsilon

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Comments: Held a Teaching Fellowship at Virginia Polytechnic Institute for the years of 1949 - 1950 and 1950 - 1951. Was employed by Hercules Powder Company, Radford Virginia from June, 1951 until September, 1952. Enrolled at the University of North Carolina Medical School in September, 1952.

W. R. Stafford, Jr.