THE OXIDATION OF HYDROCARBONS BY SOIL BACTERIA

I. MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF A SOIL DIPHTHEROID UTILIZING HYDROCARBONS

By J. N. LADD*

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Summary

(1) A description is given of the morphology and biochemical properties of a soil bacterium, *Corynebacterium* sp., able to oxidize aliphatic hydrocarbons.

(2) The rates of oxidation of *n*-hexadecane, *n*-tetradecane, *n*-decane, *n*-octane, 1-hexadecene, 1-tetradecene, and 1-dodecene are measured. R.Q. values for the oxidation of *n*-hexadecane, *n*-tetradecane, and *n*-decane indicate their complete oxidation.

(3) Increased dispersion of *n*-decane by surface-active agents did not increase its oxidation rate. However, an emulsion of *n*-decane, homogenized in a blendor and containing only one-fifth of the amount of hydrocarbon, is oxidized at the same rate as the non-homogenized control.

(4) The organism oxidizes *n*-fatty acids (C_1-C_{10}), *n*-fatty alcohols (C_2-C_{11}), several aliphatic aldehydes, and the higher methyl ketones. It is unable to oxidize the lower ketones or cyclic compounds.

(5) Co-oxidation rates of *n*-decane with its oxidative derivatives show no summation of the respective individual rates.

(6) *n*-Decane oxidation is unaffected by malonate in concentrations causing 50 per cent. inhibition of succinate oxidation. *n*-Decane oxidation is inhibited 76 per cent. by 0·004M fluoroacetate, 100 per cent. by 0·008M cyanide, 90 per cent. by 0·12M azide, 100 per cent. by 0·0001M mercuric ions, and 88 per cent. by 0·0008M iodoacetate. Cysteine (0·008M) completely reactivates cells inhibited by iodoacetate.

(7) Two non-volatile acids, chromatographically identical with lactic and glutaric acids, are produced during the oxidation of *n*-decane. No volatile acids were detected.

I. INTRODUCTION

Despite the repeated demonstration of oxidation of aliphatic hydrocarbons by a wide variety of microorganisms (Zobell 1950), little is known about the mechanism(s) involved.

Johnson, Goodale, and Turkevitch (1942) investigated the oxidation of a number of pure aliphatic hydrocarbons as well as of mixtures, by *Bacterium aliphaticum* and species of *Pseudomonas*. No intermediate oxidation products were discovered although the pH dropped from 7·0 to 6·5 in 0·001M phosphate buffer, indicative of acid production. Bushnell and Haas (1941) grew species of *Pseudomonas* and *Corynebacterium* in media containing different commercial hydrocarbons as sole sources of energy. Small decreases in pH of the culture medium and increased ease

* Division of Soils, C.S.I.R.O., Waite Agricultural Research Institute, Adelaide.

of emulsification of oil in water were assumed to be due to organic acid production during bacterial growth.

Using four strains of pathogenic mycobacteria, Saz (1949) studied the comparative rates of oxidation of the aliphatic hydrocarbons ranging from *n*-hexane to *n*-octadecane as well as their unsaturated and monohydric derivatives. Greater uptakes of oxygen were recorded for the higher homologues, but attempts to find traces of fatty acids or other intermediates failed. Webley and de Kock (1952) tested the ability of *Proactinomyces opacus* to oxidize normal fatty acids and alcohols as well as paraffins. The acids were oxidized whereas some of the lower soluble alcohols were not, due probably to their presence in toxic concentrations.

Failure to detect oxidation products other than carbon dioxide makes it impossible to decide even the carbon atom at which primary attack occurs let alone the mechanism involved. The most promising information so far comes from the work of Berhard, Gloor, and Scheitlin (1952) who fed deuterium-labelled *n*-octadecane to rats. Recovery of stearic and palmitic acids containing deuterium from the body fats and liver lipids suggests terminal oxidation.

The work presented here is a description and identification as far as possible of a soil bacterium, capable of oxidizing aliphatic hydrocarbons and their oxidative derivatives. The effect of inhibitors on hydrocarbon oxidation, and the search by chromatographic methods for end products is also described.

II. Materials and Methods

(*a*) *Isolation of Microorganism*

Isolates from Adelaide red-brown earth, previously enriched with kerosene and sump oil, were made by plating soil dilutions on a synthetic medium containing kerosene as the sole source of available carbon. The medium had the following composition: 10 ml kerosene, 3·0 g NH_4NO_3, 1·5 g KH_2PO_4, 0·25 g $MgSO_4{\cdot}7H_2O$, 0·1 g $CaCl_2{\cdot}2H_2O$, trace amounts of Fe^{++}, Zn^{++}, Cu^{++}, Mn^{++}, and MoO_4^{--} ions, 15 g agar, made up to 1 l. with distilled water pH 7·2. The inoculated plates were incubated at 25°C for 3 days. Individual colonies were passaged through the kerosene-agar until a pure culture was obtained. Stock cultures were kept on kerosene-agar at 4°C. Of a number of isolates, the microorganism selected for study gave the best compromise between rate of growth and ability to oxidize hydrocarbons.

(*b*) *Morphology*

Coverslip impressions were taken at intervals from nutrient-agar cultures incubated in a moist chamber at 28°C by the technique of Klieneberger-Nobel (1950). Photomicrographs were recorded with an Officine Galileo Universal camera microscope using the phase-contrast lens under oil immersion and an O.G. green filter (500 mμ). Either Kodak Panatomic X or Ilford HP3 film was employed. Screen magnification was ×1900. The bacterial films were fixed in Bouin's solution and stained with simple Giemsa, or by the technique of Dyar (1947), or of Webb (1954) to detect cell walls. With Dyar's technique better photographs were obtained by omitting to counterstain with methylene blue.

Biochemical tests were made according to Jensen (1934) except the sugar-fermentation experiments which were performed by the more sensitive methods of Clarke and Cowan (1952).

Suspension for respiration studies were grown in flasks of nutrient broth, pH 7·0, for 2-3 days at 26°C on a shaking machine. The cells were harvested by centrifugation, washed twice, and suspended in distilled water. The final suspension usually contained approximately 12·0 mg cell dry wt./ml (hot-air oven, 80°C for 8 hr, cooled in vacuum desiccator), or 1·1 mg total cell N/ml (semi-micro-Kjeldahl digestion). The cell suspensions maintain their original activity after storage for 2 weeks at 4°C.

Respiratory exchanges were measured by the Warburg technique at 25°C, the final volume of each cup contents being 2·5 ml. Because of the immiscibility of the hydrocarbons, they were placed in the main compartment, and the suspension (0·5 ml) added from the side-arm. For the measurement of R.Q. values, three manometers were used for each determination (Umbreit, Burris, and Stauffer 1949). Q_{O_2} values are expressed as μl O_2 consumed/mg cell N/hr.

The extraction and chromatographic procedures for the identification of non-volatile acids were the same as described earlier (Ladd and Nossal 1954). Two solvents were employed for development of paper chromatograms: Alkaline solvent—8 vol. 95 per cent. ethanol, 1 vol. water, 1 vol. 7·5N NH_4OH. Acidic solvent—water-poor phase from 3 vol. *n*-butanol, 6 vol. *iso*butanol, 3 vol. 90 per cent. formic acid, 9 vol. water.

All substrates used in this investigation were purchased from L. Light & Co., except the aliphatic monocarboxylic acids which were obtained from the British Drug Houses Ltd.

III. Results

The bacterium is a member of the saprophytic soil diphtheroids described by Jensen (1934), i.e. *Corynebacterium* sp. The organism undergoes distinct morphological changes during its life-cycle (see Plates 1 and 2). After 8-14 hr incubation in fresh medium, the old cells enlarge to long filaments which show occasional branching. The filaments appear to be multicellular, revealed particularly by Webb's stain. By 24 hr the organism has reached the logarithmic phase of growth with shorter cells occurring in the typical V formation associated with the *Corynebacterium* genus. The bacterial cells continue to decrease in length so that in the decline phase (126 hr) the organisms resemble cocci. At this time a small amount of secondary growth occurs leading to longer cells swollen at one end. The organism is non-motile, Gram-positive in young and old cells, and non-acid-fast even to 1 per cent. acid.

The *Corynebacterium* grows well on asparagine-, glycerine-, nutrient-, dextrose-, and Sabouraud's agar. After 5-6 days incubation at 28°C, the growth is smooth, glistening, and pink. A nutrient-broth culture is uniformly turbid with a fine pink sediment. It uses peptone, asparagine, nitrate, or ammonium phosphate as sources of nitrogen, is catalase positive, reduces nitrate to nitrite, and liquefies gelatine. It does not produce indole from peptone, is unable to hydrolyse starch, or invert sucrose. Acid is produced from glycerine and to a lesser extent from galactose, xylose,

and possibly glucose. No acid is produced from maltose, lactose, sucrose, raffinose, mannitol, or dulcitol.

The oxidation rates of saturated and unsaturated aliphatic hydrocarbons by the suspension are shown in Figure 1. Increase in chain length of the saturated compounds increases their rate of oxidation. This is consistently true even for *n*-hexadecane although here the initial rate fell off rapidly after 20 min incubation. *n*-Hexane and *n*-heptane were tested as substrates but manometric readings were made unreliable

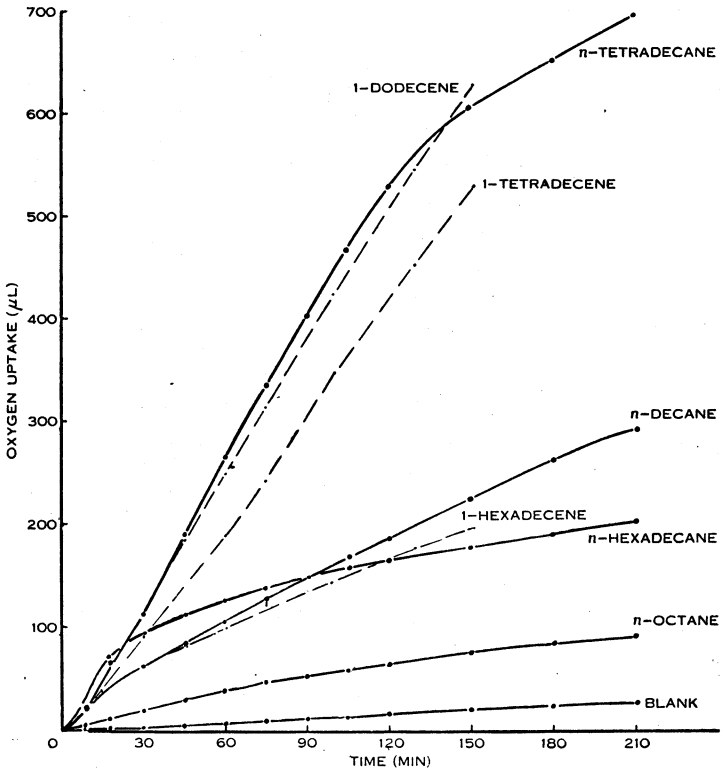

Fig. 1.—Oxidation of saturated and unsaturated aliphatic hydrocarbons. 0·1 ml of each hydrocarbon, 0·03M phosphate buffer pH 7·0, suspension 0·41 mg cell N, cup volume 2·5 ml, bath 25°C.

by their high vapour pressures. *n*-Octadecane remains in the solid state at 25°C, so its rate of oxidation cannot be strictly compared with those of its lower liquid homologues. Over a 5-hr oxidation period, an average Q_{O_2} (N) value of 50 was obtained for this hydrocarbon.

The hydrocarbons are oxidized without a preliminary lag phase but the oxidation rate is not constant. The initial rate of oxidation exceeds the secondary rate to different degrees depending upon the hydrocarbon. Of the saturated hydrocarbons, this is most marked with *n*-hexadecane and least with *n*-octane. Although not shown in Figure 1, the decline in oxidation rate is true also for the unsaturated derivatives.

Increase in the amount of hydrocarbon increases the oxygen consumption over the range tested (0·1-0·5 ml hydrocarbon per cup). The initial and secondary oxidation rates remain the same but the oxidation period at the higher rate increases with the amount of substrate added.

In contrast with the saturated hydrocarbons, the 1, 2-unsaturated derivatives show a decrease in their rate of oxidation with increasing chain length, except 1-octene which is not oxidized and even inhibits endogenous respiration.

(*a*) *Use of Dispersing Agents*

Because of the insolubility of the hydrocarbons, the use of dispersing agents to promote greater substrate surface for biological attack was investigated. "Tween 80" is itself oxidized by the suspension at a rate greater than that of *n*-decane. Correction for oxidation of the detergent revealed that it neither stimulates nor inhibits *n*-decane oxidation. Sodium desoxycholate is not oxidized, and in a concentration of 0·015 per cent. promotes increased dispersion of *n*-decane. However, hydrocarbon oxidation is not increased.

Emulsions of *n*-decane in buffer were made by homogenizing in a Waring Blendor for 2 min. Although in no case did the emulsions promote greater uptakes of oxygen than an equal amount (0·1 ml) of non-emulsified hydrocarbon, yet equal consumptions were obtained with emulsions containing one-fifth of this amount per cup. This indicates that the enzyme system oxidizing *n*-decane is saturated by 0·1 ml non-emulsified *n*-decane or by 0·02 ml when emulsified. The observed increase in oxygen consumption with increasing amounts of non-emulsified *n*-decane as stated earlier is therefore hard to explain, although it should be pointed out that the actual oxidation rates remain identical.

(*b*) *Oxidation of Cyclic Compounds*

The suspension was tested for its ability to oxidize aromatic hydrocarbons (benzene, toluene, *o*-xylene, *p*-xylene), saturated ring structures (*cyclo*hexane), and partially saturated cyclic hydrocarbons (tetralin), but in no case was a net oxygen uptake observed.

(*c*) *R.Q. Values of the Saturated Hydrocarbons*

The calculated R.Q. values for the oxidation of *n*-decane, *n*-tetradecane, and *n*-hexadecane by the suspension in 0·025M phosphate buffer, pH 7·0, are 0·69, 0·62, and 0·65 respectively. The theoretical R.Q. value for the complete oxidation of a saturated aliphatic hydrocarbon is 0·65.

(*d*) *Oxidation of Saturated Aliphatic Alcohols*

The rates of oxidation of the normal saturated monohydric alcohols from ethanol to *n*-undecyl alcohol are shown in Figure 2. The solubility of *n*-octyl, *n*-nonyl, *n*-decyl, and *n*-undecyl alcohols was so low that they were added to the manometer cups in the same manner as the hydrocarbons. The lower homologues were added as 0·01M

aqueous solutions. Good oxygen uptakes were obtained with all the alchohols, except *n*-butanol which is poorly oxidized.

(*e*) *Oxidation of Saturated Aliphatic Carbonyl Compounds*

Four aldehydes only were available for testing, viz. acetaldehyde and its insoluble C_6, C_8, and C_{10} homologues. The latter three compounds were added to the cups in the same manner as the hydrocarbons. Acetaldehyde was readily oxidized without a lag period, giving a Q_{O_2} (N) value of 160, averaged over an experimental

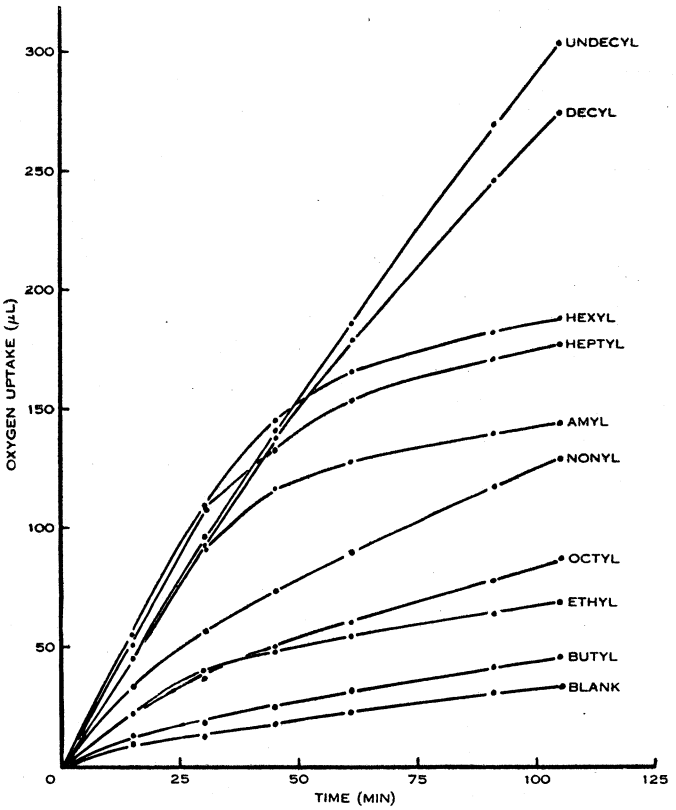

Fig. 2.—Oxidation of saturated monohydric alcohols. 0·1 ml of octyl, nonyl, decyl, and undecyl alcohols, and 0·1 ml of 0·01M solutions of the lower alcohols, 0·02M phosphate buffer pH 7·0, suspension 0·68 mg cell N, cup volume 2·5 ml, bath 25°C.

period of 3 hr. *n*-Hexyl aldehyde and *n*-octyl aldehyde both inhibited the endogenous respiration of the suspension. *n*-Decyl aldehyde is not oxidized consistently. Frequently inhibition of blank respiration occurred, yet net oxygen uptakes have been obtained giving a Q_{O_2} (N) value of 80 over a 3-hr incubation period. In contrast, acetone and methyl ethyl ketone are poorly oxidized, whereas excellent oxygen uptakes are obtained with methyl nonyl ketone. Using the same suspension whose

endogerous respiration is inhibited by *n*-decyl aldehyde, a Q_{O_2} (N) value of 445 was obtained for metnyl nonyl ketone averaged over an incubation period of 2 hr.

(*f*) *Oxidation of Saturated Aliphatic Acids*

The rates of oxidation of the normal saturated monocarboxylic acids from formic to capric by the suspension are shown in Figure 3. Fatty acids, either with odd or even numbers of carbon atoms, are oxidized at the same rate without a lag period. For a given acid, the total oxygen uptake varies with the suspension, although good correlation is obtained between duplicate cups. In all cases, oxygen consumption was less than required for complete oxidation.

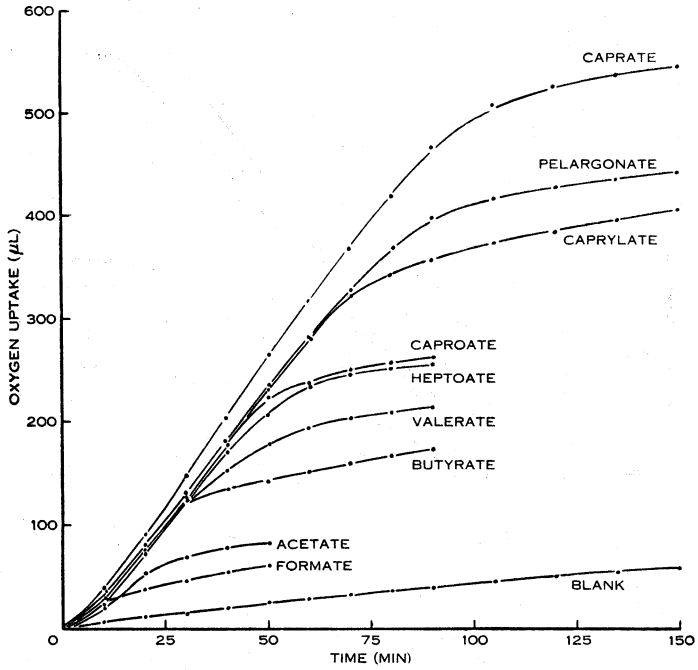

Fig. 3.—Oxidation of saturated monocarboxylic acids. 0·2 ml of 0·01M acid (sodium salt), 0·03M phosphate buffer pH 7·0, suspension 0·41 mg cell N, cup volume 2·5 ml, bath 25°C.

(*g*) *Co-oxidation of* n-*Decane with its Derivatives*

n-Decane was co-oxidized with *n*-decyl alcohol, *n*-decyl aldehyde, and *n*-decylic acid. The results are shown in Figure 4. The rate of oxidation of *n*-decane and *n*-decylic acid as co-substrates equals that of the faster oxidized individual compound (*n*-decylic acid). This proceeds until depletion of the fatty acid when the co-oxidation rate decreases to that of *n*-decane alone.

The oxidation rate of *n*-decane plus *n*-decyl alcohol exceeds the rate of oxidation of the individual compounds but is far less than the sum of the individual rates. The co-oxidation rate equals the initial high rate of *n*-decane oxidation and maintains

this rate after *n*-decane oxidation decreases. Co-oxidation of *n*-decane plus *n*-decyl aldehyde proceeds at a rate intermediate between the individual rates.

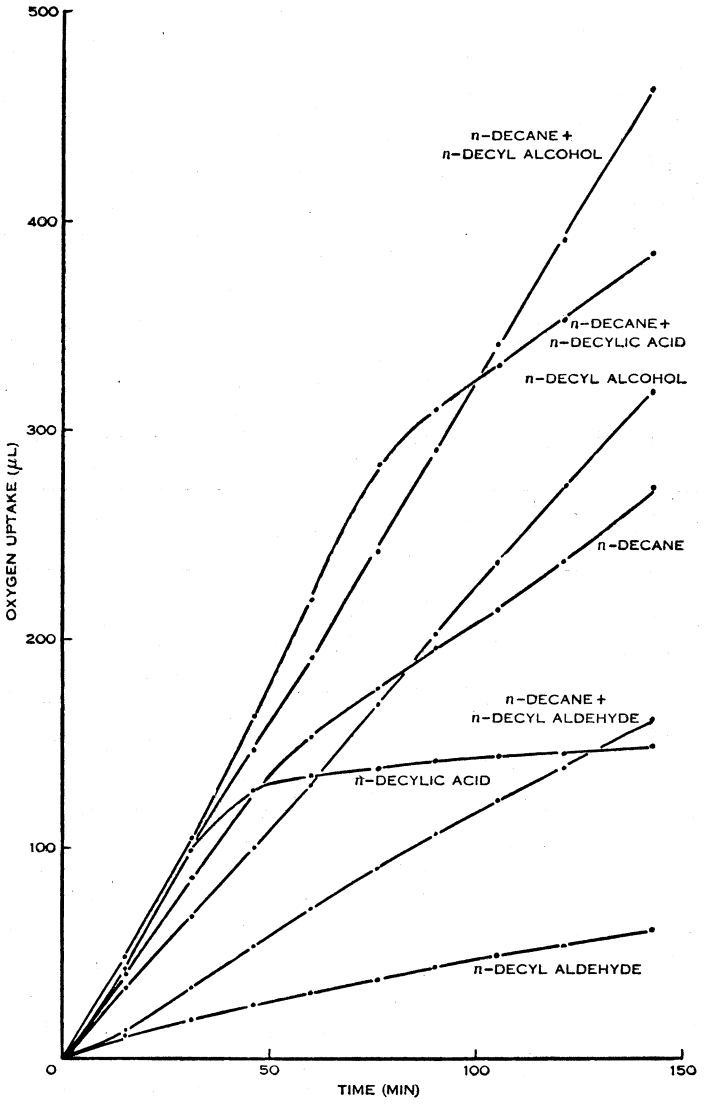

Fig. 4.—Co-oxidation of *n*-decane with its oxidative derivatives, corrected for endogenous respiration. 0·1 ml of each substrate except decylic acid which was added as a 0·01M solution, 0·03M phosphate buffer pH 7·0, suspension 0·27 mg cell N, cup volume 2·5 ml, bath 25°C.

(*h*) *The Effect of Inhibitors on* n-*Decane Oxidation*

The effects of various inhibitors on the oxidation of *n*-decane by the suspension are shown in Table 1. Experiments were carried out in cups containing 0·1 ml of hydrocarbon in 0·025M phosphate buffer at pH 7·0, except in the case of malonate where the pH was 6·0.

At pH 6·0, oxidation of 0·016M succinate was inhibited 50 per cent. by 0·024M malonate, but at this pH and concentration, malonate had no effect on *n*-decane oxidation. Fluoroacetate in concentrations of 0·02 and 0·004M inhibited the rate of oxidation of 0·004M sodium acetate by 66 and 38 per cent. respectively. Both concentrations inhibit *n*-decane oxidation 76 per cent. The initial fast rate of *n*-decane oxidation is unaffected by fluoroacetate which inhibits the secondary steady rate only.

In contrast, cyanide and azide appear to inhibit the initial rate only. The percentage inhibitions as tabulated for these compounds are functions of the period of oxidation. Cyanide (0·0008M) has no effect on *n*-decane oxidation. In the presence

TABLE 1

THE EFFECT OF INHIBITORS ON *n*-DECANE OXIDATION

Inhibitor	Final Concentration	Cell N (mg/cup)	Percentage Inhibition
Malonate	0·024M	0·44	0
Fluoroacetate	0·004M	0·47	76
	0·02M	0·47	76
Cyanide	0·0008M	0·32	0
	0·0016M	0·32	50
	0·0048M	0·32	75
	0·008M	0·32	100
Azide	0·04M	0·32	0
	0·08M	0·32	72
	0·12M	0·32	90
Mercuric chloride	0·00001M	0·30	23
	0·0001M	0·30	100
Iodoacetate	0·0008M	0·30	88
Iodoacetate +cysteine	0·0008M 0·008M	0·30	0

of 0·0016M cyanide, there is no preliminary high rate of oxidation. The oxygen uptake remains constant throughout and equal to the secondary rate in the absence of inhibitor. This is again observed in the presence of 0·0048M cyanide, but here there is a lag period before oxidation begins.

The sulphydryl reagents inhibit both the initial and secondary oxidation rates. Cysteine reactivated both rates inhibited by iodoacetate.

(*i*) *Search for Products of* n-*Decane Oxidation*

n-Decane (0·1 ml) was added to a suspension of the organisms in a 100-ml conical flask and shaken at 25°C for varying times, after which the cup contents were

brought to pH 1 with 5N H_2SO_4. An equivalent amount of suspension without the hydrocarbon was treated in the same manner.

(i) *Volatile Acids.*—The acidified flask contents were steam distilled and the distillate collected in standardized sodium hydroxide. Excess alkali was back-titrated with standard HCl using phenolphthalein as indicator. In three separate experiments, the control suspensions contained 1·74, 4·42, and 0·61 μ-equiv. of volatile acid per mg cell nitrogen respectively. However, no increase in volatile acid occurs during oxidation of *n*-decane.

TABLE 2

NON-VOLATILE ACID CONTENT OF SUSPENSIONS OXIDIZING *n*-DECANE

Suspension No.	Cell N (mg/cup)	Incubation Period (hr)	Non-volatile Acid (μ-equiv.)			
			Experimental		Blank	
			Acid 1	Acid 2	Acid 1	Acid 2
1	13·4	3	2·20	1·82	3·01	1·76
2	4·4	4	1·54	0·92	1·76	1·04
3	19·5	18	1·69	3·20	1·04	0·46

(ii) *Non-volatile Acids.*—Only two acids were discovered in the bacterial extracts but these were not positively identified. One acid ran identically on column and paper chromatograms with glutaric acid, the other with lactic acid, but no further identification was made. The results of several determinations are tabulated (Table 2). The corresponding peaks from the experimental and blank columns ran identically as single spots on two-dimensional paper chromatograms. The first and second column peaks respectively gave R_F values of 0·27 and 0·54 in the alkaline solvent and 0·86 and 0·75 in the acidic solvent.

IV. DISCUSSION

Although none of the described species has morphological and biochemical properties completely identical to those of the organism studied, it is a member of the *Corynebacterium* genus (Jensen 1934; Bergey 1948). The organism belongs to the large sub-group which includes the saprophytic, soil-inhabiting diphtheroids, the properties of which are reviewed by Jensen (1952). Conn and Dimmick (1947) have suggested that many members of this group be classified in a separate genus, *Arthrobacter*, the type species of which is *A. globiforme*, a typical soil organism. This genus is characterized by marked changes in cell morphology during its life-cycle, ability to utilize simple inorganic sources of nitrogen, weak fermentative ability, and a tendency to be Gram-negative in young cultures but changing to Gram-positive in older cells. The bacterium studied possesses all of these properties, except that it is Gram-positive even in young cultures.

The ability to oxidize hydrocarbons is common to many groups of bacteria but it has not been shown to be a property widespread in the genus *Corynebacterium*. In fact Haag (1927) used the inability of corynebacteria to utilize paraffin as a means of differentiation of this class from the mycobacteria. Later, Bushnell and Haas (1941) showed that *C. simplex* and several unidentified and undescribed species of corynebacteria could grow in mineral media to which solid paraffin had been added. Only *C. simplex* utilized kerosene as a sole source of carbon. The bacterium under investigation differs from *C. simplex* in a number of properties and appears to be another member, previously undescribed, of this group, capable of actively metabolizing aliphatic hydrocarbons.

Very little positive information is available on the position of attack and mechanism of biological oxidation of aliphatic hydrocarbons. The lack of polar and ionic groups in the hydrocarbon molecule suggests that the first step in the oxidation is one of activation, due perhaps to the formation of free radicles.

Leach (1954) has recently reviewed the theories of mechanism of enzymatic oxido-reduction. Non-enzymatic autoxidation of hydrocarbons (RH) is initiated by the formation of the free radicle $R^{\cdot}$ which combines with oxygen to form the peroxide $RO_2^{\cdot}$. Further reaction of the peroxide with another hydrocarbon molecule results in the hydroperoxide and regeneration of a second $R^{\cdot}$ radicle, which repeats the process, thus

$$RO_2^{\cdot} + RH \rightarrow RO_2H + R^{\cdot}.$$

Thiyl radicles may be important in the initial activation. Bickel and Kooijman (1952) have shown that reactions of the type

$$RH + R_1S^{\cdot} \rightarrow R^{\cdot} + R_1SH$$

may be reversible at room temperatures and suggest their possible role in biological oxidations. The importance of the sulphydryl group in its association with enzymes responsible for oxido-reductions has been pointed out by Barron (1951). We have shown that *n*-decane oxidation is markedly inhibited by sulphydryl reagents, iodoacetate, and mercuric chloride, but that normal oxidation is fully restored by the addition of cysteine.

From an analysis of the end products of *n*-decane autoxidation at 145°C, Benton and Wirth (1953) concluded that *n*-decane is primarily attacked equally well at carbon atoms 2, 3, 4, and 5, but that terminal oxidation is negligible. In contrast, Berhard *et al.* (1952) have isolated stearic and palmitic acids containing deuterium from the body fat of rats fed on deuterium-labelled *n*-octadecane, suggesting oxidation at the terminal carbon atom. Whether the discrepancy observed between the positions of primary attack of hydrocarbons, autoxidized or by enzymatically controlled reactions, reflects basically different mechanisms of oxidation is still in doubt.

If the hydroperoxide were formed from the biological oxidation of the aliphatic hydrocarbons, then peroxidatic activity could result in the formation of the corresponding fatty alcohol which in turn could be oxidized to the fatty acid. This may then be oxidized by β-oxidation to carbon dioxide and water.

R.Q. measurements suggest that the hydrocarbon is completely oxidized. The lack of specific inhibitors and the ready oxidation of possible intermediates prevent

an accumulation of the latter. The inability to demonstrate volatile acid formation from *n*-decane oxidation may be explained on this basis.

The ready oxidation of methyl nonyl ketone contrasted with the poorer and inconsistent oxidation of *n*-decyl aldehyde, suggests that oxidation of the hydrocarbon may proceed at a position other than the terminal carbon atom. However, it is doubtful whether the possible intermediates, tested for their ability to be oxidized by the suspension, really exist as such when produced enzymatically from the hydrocarbon. More probably they exist as activated forms as is the case for the oxidation of the fatty acids themselves. The non-oxidation of a suspected intermediate may be misleading due to an inability to form the activated derivative from the parent compound, yet, nevertheless, may be well oxidized as the activated form produced from the hydrocarbon itself.

Chromatographic analysis has revealed the formation of at least two non-volatile acids from *n*-decane oxidation. However, the same two acids are formed during growth of the organisms in nutrient broth. Production from *n*-decane was only demonstrated by depletion of the endogenous substrates by long incubation. It seems likely that the two acids are members of the general degradative pathway employed by the organisms and are not produced specifically from *n*-decane oxidation. The acids are chromatographically identical with lactic and glutaric acids but further identification has not been attempted.

Co-oxidation of *n*-decane with its oxidative derivatives, shows that in no case does the co-oxidation rate equal the sum of the individual rates. This indicates a common metabolic pathway, or at least the involvement of common group-specific enzymes at some stage in the degradation of the substrates.

V. Acknowledgments

I would like to thank Dr. R. J. Swaby for helpful discussions and Miss Grace Banos for technical assistance. I am especially indebted to Mr. J. R. Harris for staining and photographing the bacterial preparations.

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EXPLANATION OF PLATES 1 AND 2

All preparations are coverslip impressions of the growth of *Corynebacterium* sp. on nutrient agar. Magnification × 1900.

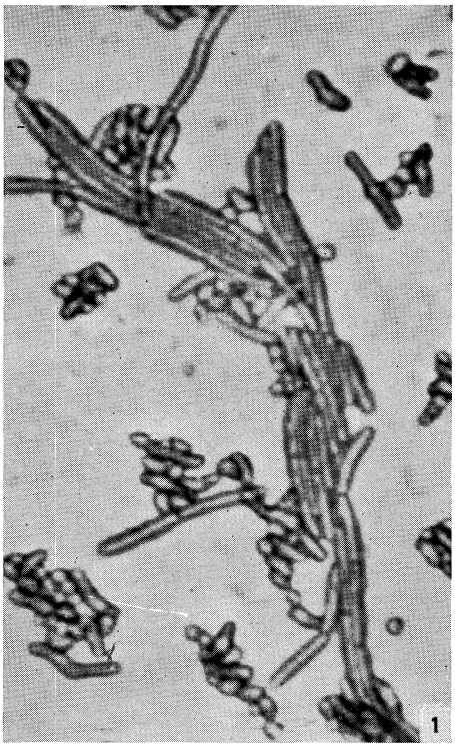
PLATE 1

Fig. 1.—8-Hr culture. Dyar cell-wall stain.
Fig. 2.—8-Hr culture. Webb cell-wall stain.
Fig. 3.—14-Hr culture. Giemsa stain showing occasional branching of the filaments.
Fig. 4.—24-Hr culture. Giemsa stain.

PLATE 2

Fig. 1.—24-Hr culture. Webb cell-wall stain.
Fig. 2.—126-Hr culture. Dyar cell-wall stain.
Fig. 3.—126-Hr culture. Dyar cell-wall stain showing early stages of secondary growth.

THE OXIDATION OF HYDROCARBONS BY SOIL BACTERIA. I



THE OXIDATION OF HYDROCARBONS BY SOIL BACTERIA. I