Cultivation of the Yeast *Candida lipolytica* on Hydrocarbon

III. Oxidation and Utilization of Individual Pure Hydrocarbons

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ABSTRACT. The ability of the yeast *Candida lipolytica* 4-1 to oxidize and utilize various pure aliphatic hydrocarbons occurring in gas oil was studied. It was found that the given strain of *Candida lipolytica* oxidized n-alkanes without adaptation, starting with heptane, and utilized them for growth, starting with nonane. Isoalkanes with a single methyl group in the side chain were also oxidized and utilized for growth, but less than the corresponding n-alkanes. The site of the methyl group in the isoalkane chain influences its conversion to biomass. Branched chains at both ends of the isoalkane molecule prevent its utilization for growth of *Candida lipolytica*. 1-olefines are also oxidized and utilized for growth, though less than the corresponding n-paraffins. Alkylaromatic hydrocarbons are oxidized from amylbenzene up to decylbenzene, which is utilized only slightly for growth of the yeast.

Gas oil containing 10—20% n-alkanes, or paraffins isolated from gas oil, are a suitable carbon source for the cultivation of yeasts of the genus *Candida* (Champagnat *et al.*, 1963a, b). In cultivation of the yeast *Candida lipolytica* on gas oil medium, we found (Dostálek *et al.*, 1968a) that the first degraded were n-alkanes with a molecule containing a small number of carbon atoms (C_{10} to C_{17}), while those with a large number (C_{18}—C_{25}) were utilized in the later phase of fermentation. During the degradation of higher n-alkanes the freezing point of gas oil decreased rapidly without a corresponding biomass increase.

The aim of this study was to verify whether the data obtained in a highly complex substrate also applied to the use of individual pure n-alkanes. In addition, we investigated the relationship between the oxidization and utilization of other types of hydrocarbons by *Candida lipolytica*. We paid special attention to hydrocarbons present in gas oil in significant quantities, e.g. alkylaromatic hydrocarbons, slightly branched isoparaffins and 1-olefines, as any oxidation of these substances, without utilization, could lead to serious deterioration of the quality of biologically deparaffinized gas oil.

MATERIALS AND METHODS

Microorganism. We worked with the strain *Candida lipolytica* 4-1 used in previous studies (Dostálek *et al.*, 1968a, b). This strain was isolated in the oil-fields of Southern Moravia and was adapted by continuous cultivation until it was resistant to a high sulphur compound concentration (1.5—2% sulphur) in gas oil and grew on completely inorganic medium containing hydrocarbons, without the addition of organic nitrogen

compounds. The strain *Candida lipolytica* 4-1 is maintained by regular passage on wort agar slants.

Nutrient media. For studying the rate of oxidation of individual pure hydrocarbons we prepared a *Candida lipolytica* 4-1 culture by cultivation on inorganic medium containing glucose: glucose — 25 g; KH_2PO_4 — 7 g; $MgSO_4$ — 0.2 g; NaCl — 0.1 g; NH_4Cl — 4.5 g; tap water — up to 1,000 ml; pH — 5.

The medium was poured in amounts of 50 ml into 500-ml culture flasks and was sterilized in an autoclave at 0.8 kp/$/cm^2$ for 20 minutes.

The composition of the growth medium for studying utilization of the various hydrocarbons was the same as above, except that glucose was replaced by the relevant hydrocarbon, which was added to the flasks under non-sterile conditions in amounts of 0.5 ml/50 ml nutrient medium.

If hydrocarbons are solid at normal temperature, we first dissolved them in squalane, which is known not to be oxidized by *Candida lipolytica*. The concentration of the test hydrocarbon in the nutrient medium was 1%.

Cultivation methods

Preparation of *Candida lipolytica* 4-1 suspension for measuring the rate of hydrocarbon oxidation

Culture flasks containing nutrient medium were inoculated with a culture from a wort agar slant and were cultivated on a reciprocating shaker apparatus at 30° C. After 24 hours the yeast cells were centrifugated washed with 2/15 M phosphate buffer at pH 6 and resuspended in glucose-free mineral medium. The suspension was again shaken at 30° C to ensure complete dispersion of the residual carbon substrate. After 2—3 hours the yeast cells were again centrifug ed, washed twice with phosphate buffer and resuspended in the same buffer to give a concentration of 2—3 mg cells/ml suspension. This limited endogenous respiration of the culture, while preserving its oxidative activity for hydrocarbons.

Preparation of *Candida lipolytica* 4-1 suspension for measuring the rate of synthesis of biomass

Inoculum was prepared similarly to the yeast cell suspension for measuring the rate of hydrocarbon oxidation. Young (24-hour) cells grown on glucose medium were washed with buffer, starved, washed again and suspended in buffer to form a dense suspension containing 3—4 mg yeast cell dry weight/ml. The whole process was performed under sterile conditions. Flasks with medium containing the individual test hydrocarbons were inoculated with 1—2 ml inoculum and cultivated on a reciprocating shaker apparatus at 30° C. Evaporation of some low-boiling hydrocarbons was prevented by incubating at slight atmospheric overpressure. Each culture flask was sealed with a rubber stopper pierced by a glass tube connected by rubber tubing, and cotton wool filter, with a 20-liter glass bottle sealed by a rubber stopper with two perforations. The second inlet was connected with the outflow of another bottle higher than the first one and filled with water. Any decrease in overpressure in the culture flasks was automatically compensated by an inflow of water from the higher bottle into the empty bottle connected with the culture flask. Samples were collected at given intervals to determine dry weight, residual nitrogen and the pH.

Analytical methods

Determination of rate of hydrocarbon oxidation

The rate of oxidation of hydrocarbons by a resting *Candida lipolytica* 4-1 culture was measured manometrically in a Warburg apparatus at 30° C. We used Warburg vessels with one side arm and

Table 1. Scheme of preparation technique and purity characteristics of hydrocarbon standards employed

Hydrocarbon	Preparation technique	Boiling point (°C)		Solidif. point (°C)		n_D^{20}		Purity
		finding	tab. vaule	finding	tab. value	finding	tab. value	(% hm)
Pentane	Rectification of pure pentane (Lachema)	36.1	36.07			1.3570	1.3575	99.90
Hexane	Propylbromide-Wurtz reaction	68.7	68.75			1.3747	1.3749	99.90
Heptane	Rectification of pure heptane (Lachema)	98.5	98.43			1.3885	1.3877	99.90
Octane	Hydrogenation of octylalcohol on MoS_2	125.5	125.67			1.3978	1.3974	99.95
Nonane	Butylbromide + ethyl formate Mg 5-nonanol-dehydration-hydrogenation	150.7	150.77			1.4053	1.4054	99.95
Decane	Amylbromide-Wurtz reaction	174.2	174.12			1.4113	1.4118	99.90
Undecane	Octylbromide + allylbromide Mg 1-undecenehydrogenation	195.8	195.84			1.4170	1.4172	99.90
Dodecane	Hexylbromide-Wurtz reaction	216.3	216.27			1.4213	1.4216	99.95
Tridecane	Decylbromide + allylbromide Mg 1-tridecenehydrogenation	235.5	235.50			1.4224	1.4224	99.95
Tetradecane	Heptylbromide-Wurtz reaction	253.5	253.60	5.5	5.50	1.4288	1.4291	99.40
Pentadecane	Heptylbromide + ethyl formate Mg 5-pentadecanol-dehydration-hydrogenation	270.2	270.6	9.8	9.81	1.4312	1.4319	99.80
Hexadecane	Hydrogenation of hexadecylalcohol on MoS_2	287.0	287.1	18.1	18.14	1.4349	1.4345	99.50
Heptadecane	Octylbromide + ethyl formate Mg 9-heptadecanol-dehydration-hydrogenation	302.3	302.7	21.3	21.72	1.4360	1.4368	98.00
Octadecane	Hexadecylbromide + acetaldehyde Mg 2-octadecanol-dehydration-hydrogenation	316.6	317.50	27.5	28.10	—	—	97.50
Nonadecane	Hexadecylbromide + allylbromide Mg 1-nonadecene-hydrogenation	227.0/50 torr	228.0/50 torr	31.8	32.00	—	—	99.50
Eicosane	Decylbromide-Wurtz reaction	204.0/15 torr	205.0/15 torr	36.5	36.7	—	—	99.70
Heptene-1	Dibutylmagnesium bromide + allylbromide-rectification	93.60	93.64			1.3996	1.3998	99.85
Octene-1	n-amylmagnesium bromide + allylbromide-rectification	121.30	120.28			1.4090	1.4087	99.90
Decene-1	n-heptylmagnesium bromide + allylbromide-rectification	170.65	170.57			1.4214	1.4215	99.80
Undecene-1	n-octylmagnesium bromide + allylbromide-rectification	107.0/50	106.87/50			1.4259	1.4261	99.95
Trideceno-1	n-decylmagnesium bromide + allylbromide-rectification	103.0/10	102.5/10			1.4340	1.4336	99.90
Tetradecene-1	n-undecylmagnesium bromide + allybromide-rectification	124.0/15	124.5/15			1.4362	1.4365	99.80

Hexadecene-1	n-hexadecylalcohol + palmitic acid — degradation of palmitate — rectification — preparative chromatography (Kraft, 1883; Canin *et al.*, 1954)	207.0/100	205.3/100			1.4405	1.4410	98.20
Octadecene-1	Product of Aldrich Chemical Co., Inc., U.S.A.	186.5/20	186.0/20			1.4445	1.4449	98.70
Nonadecene-1	n-hexadecylmagnesium bromide + allylbromide-rectification	176.0/10	177.2/10	21.4	21.7	—	—	—
Ethylbenzene	Rectification of industrial product	136.2	136.19			1.4957	1.4954	99.80
Propylbenzene	Bromobenzene + propylbromide	159.0	159.22			1.4923	1.4920	99.95
Butylbenzene	Bromobenzene + butylbromide	184.2	183.85			1.4892	1.4897	99.85
Isobutylbenzene	Benzene + isobutyric chloride $\underline{FeCl_3}$ isopropyl — phenylketane — Wolff-Kizhner reduction	173.0	172.85			1.4860	1.4865	98.50
Sec. butylbenzene	Ethylbromide + acetophenone $\underline{Mg}$ 2-phenyl-2-butanol — dehydration — hydrogenation	173.0	173.30			1.4893	1.4902	98.20
Tert. butylbenzene	Benzene + tert. butylchloride on $AlHg_x$ (Diuguid, 1941)	169.5	169.50			1,4925	1.4926	99.95
Amylbenzene	Bromobenzene + amylbromide	205.0	205.30			1.4870	1.4884	99.10
Hexylbenzene	Bromobenzene + hexylbromide	228.3	227.35			1.4880	1.4888	98.50
Heptylbenzene	Bromobenzene + heptylbromide	238.2	237.70			1.4877	1.4884	96.10
Octylbenzene	Bromobenzene + octylbromide	261.5	260.00			1.4848	1.4851	99.40
Decylbenzene	Bromobenzene + decylbromide	116.5/2 torr	116.0/2 torr			1.4825	1.4832	98.20
2-methyl-dodecane	Decylbromide + acetone $\underline{Mg}$ 2-methyl — 2-tridecanol — dehydration — hydrogenation (Petrov *et al.*, 1959)	103.0/10 torr	103.0/ /10.5 torr	41		1.4234	1.4235	99.80
2-methyltetradecane	Dodecylbromide + acetone $\underline{Mg}$ 2-methyl — 2-tetradecanol — dehydration — hydrogenation	265.0	265.40			1.4312	1.4304	97.20
3-methyltridecane	Decylbromide + methylethylketone $\underline{Mg}$ 3-methyl — 3-tridecanol — dehydration — hydrogenation (Petrov *et al.*, 1959)	106.0/7 torr	106.0/7 torr			1.4307	1.4307	99.95
8-methyl-pentadecane	Heptylbromide + ethyl octane $\underline{Mg}$ 8-methyl — 8-pentadecanol — dehydration — hydrogenation (Petrov *et al.*, 1959)	150.0/15 torr	150.0/15 torr			1.4341	1.4331	99.80
2,11-dimethyl-dodecane	Diethylester of sebacic acid + methyliodide $\underline{Mg}$ 2,11-dimethyl — 2,11-dodecanediole — dehydration — hydrogenation (Landa & Kejvan, 1931)	119.0/15 torr	117.0/13 torr			1.4318	—	98.75
2,13-dimethyltetra-decane	Diethylester of sebacic acid $\underline{LiAlH_4}$ 1,10-decanediole $\underline{HBr}$ 1,10-dibromodecane + + acetone $\underline{Mg}$ 2,13-dimethyl — 2,13-tetradecanediole — dehydration — hydrogenation (Buchta & Weidinger, 1953; Lukeš & Bláha, 1952)	123.0/8 torr	—			1.4328	—	98.60

a central funnel and filled them as follows: into the side arm we pipetted substrate (hydrocarbon), either concentrated (0.2 ml) or as a stable hydrocarbon emulsion in phosphate buffer (1 ml) prepared by sonication (solid hydrocarbons were predissolved in squalane). Into the central space we pipetted 1 ml of a resting yeast cell suspension (dry weight 2—3 mg) and 1.8 (or 0.8) ml 1/15 M phosphate buffer at pH 6. Into the central funnel we pipetted 0.2 ml 20% KOH and inserted a small piece of folded filter paper to ensure quicker absorption of the CO_2 formed. Oxygen consumption was read at 10-minute intervals for 1—2 hours. Absolute oxygen consumption on the individual hydrocarbons was expressed as Q_{O2} of the culture.

The dry weight of the resting yeast cell suspension was determined from the difference between the dry weight of a 1 ml yeast cell suspension in buffer and of 1 ml pure buffer, after drying in glass cuvettes at 105° C to constant weight.

Determination of increase in amount of biomass

The biomass dry weight was determined gravimetrically by filtering a measured amount of culture medium containing yeast cells through porous G_4 frit; the sediment on the frit was washed with acetone and petroleum ether so as to clear the cells of any remains of the fermentation medium still adhering to them and was then dried at 105° C to constant weight.

Ammoniacal nitrogen was determined in the fermentation medium by titration after distilling from a Markham apparatus (Markham, 1942), using an indicator according to Ma and Zuazaga (1942).

The increase in the yeast cell biomass was calculated indirectly, from the decrease in the amount of ammoniacal nitrogen in the fermentation medium during cultivation. The amount of nitrogen, multiplied by the factor 6.25, gives the amount of protein in the yeast cells. Since *Candida lipolytica* dry biomass contains about 50% protein, the amount of nitrogen consumed during fermentation, multiplied by the factor 12.5, corresponds to the amount of biomass formed in a given volume of fermentation liquid.

Changes in the pH during cultivation of the yeast cells in growth medium were controlled by measuring in an electron tube pH-meter, using a glass and a calomel electrode.

Preparation of pure hydrocarbons

Because of the large number of hydrocarbon standards prepared we give only the general outlines of the various methods of synthesis, without describing the individual techniques in detail. A survey of the preparation techniques, together with the purity characteristics of the products, is given in Table 1.

In most cases the initial raw materials were available alcohols, which were treated either directly or after conversion to the corresponding bromides by the following basic methods:

(1) by direct hydrogenation of alcohols on sulphide catalysts (Landa & Mostecký, 1955; Landa *et al.*, 1957a; Landa & Weisser, 1957b; Landa *et al.*, 1959; Landa & Weisser, 1956);

(2) by hydrogenation of olefines obtained by dehydration of alcohols (Brooks *et al.*, 1954; Edgar *et al.*, 1929) prepared by Grignard's reaction (Bláha, 1961; Grignard, 1926) on Raney's nickel;

(3) by hydrogenation of olefines prepared by Grignard's reaction from the relevant alkylmagnesium bromide and allyl bromide on Raney's nickel (Asinger, 1942; Suida & Drahowzal, 1942; Kazansky *et al.*, 1947);

(4) by the Wurtz and Wurtz-Fittig reaction (Fahim & Mustafa, 1949; Faillebin, 1924, Lavina & Shusherina, 1955; Wooster, 1932).

For synthesis of some hydrocarbons

we chose other techniques, or obtained them by chemical purification of crude hydrocarbons available on the home market.

The prepared hydrocarbons were subjected to final purification by rectification in a column 150 cm long and 1.5 cm in diameter fitted with a heating jacket and filled with stainless spirals with a triangular profile, of the Helipac type, which displayed 40 TP effectiveness. All hydrocarbons whose boiling point was lower than 320° C were rectified in this column at normal pressure. Hydrocarbons with a higher boiling point were rectified *in vacuo* in a Vigreux column of the same dimensions. The rectification products were finally treated by percolation through a JP 3 activated silica gel column.

The purity of the completed hydrocarbon standards was controlled by comparing boiling point — and for high-boiling hydrocarbons freezing point and the refractive index — with the tabular values (Obolentsev, 1953). The quantitative criterion of purity was gas chromatographic analysis done in a type D-ACI Research Chromatograph (Messrs. Carlo Erba), using an insulating detector and a capillary column with an internal diameter of 0.2 mm, moistened with Apiezon L. The nitrogen (carrier gas) flow was maintained at 0.2 ml/min, with a division ratio of 1 : 400; the hydrogen and air (auxiliary gas) inflow was maintained at 20 and 325 ml/min respectively. The temperature of the detector was 150° C and the temperature of the column was modified according to the boiling point of the given hydrocarbon. Under these conditions the HETP of the capillary column was 0.32 mm and the separation factor 170, determined for heptane at 60° C.

The purity of alkylaromatic hydrocarbons was further controlled chromatographically in a column 2 m long and with an internal diameter of 2 mm, filled with Chromosorb W (60—80 mesh) moistened with Carbowax 20 M. The aim was to confirm that these hydrocarbons were not contaminated by alkanes formed as side-products during the Wurtz-Fittig reaction, whose boiling point, in some cases, is very similar to that of alkylaromatic hydrocarbons.

The results obtained with pure synthetically prepared n-alkanes were verified by isolating individual n-alkanes of equivalent purity directly from gas oil. The group of alkanes was isolated with urea in the usual way from R-315 gas oil supplied by Messrs. Slovnaft (distillation range 210—350° C, n-alkane content 18.6% w/w). The released n-alkanes were fractionated by distillation in the 40° C range and individual pure n-alkanes were isolated from the fractions by preparative chromatography. Chromatographic separation was done in a model P Fraktovap preparative chromatograph (Messrs. Carlo Ebra) in columns 2 m long and with an internal diameter of 10 mm, filled with Chromosorb W (30—60 mesh) moistened with 10% Apiezon L (for C_9—C_{13} hydrocarbons) or with 10% methylsilicone polymer SE-30 (for C_{14}—C_{16} hydrocarbons). Two fold preparation yielded C_9—C_{16} n-alkanes of the following purity:

nonane	99.2%
decane	99.1%
undecane	99.2%
dodecane	99.3%
tridecane	98.3%
tetradecane	99.5%
pentadecane	99.4%
hexadecane	98.7%

The impurities accompanying these standards were mildly branched isoalkanes transferred during urea isolation; they had similar boiling points and could not be separated in the slightly active preparative columns.

RESULTS AND DISCUSSION

Oxidation of hydrocarbons by the yeast *Candida lipolytica* 4-1

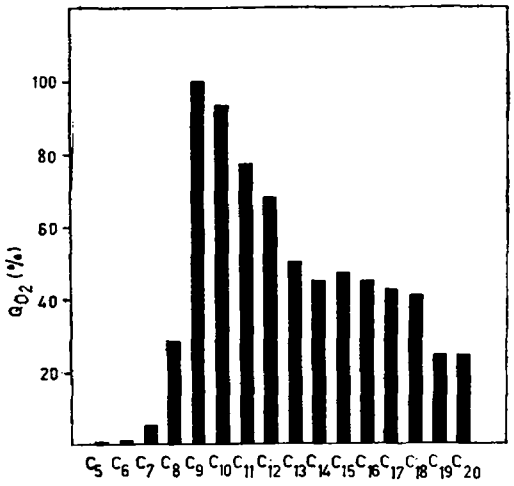

Fig. 1. Oxidation of pure n-alkanes by *Candida lipolytica* 4-1. *y*: Qo_2 values expressed in percentage form after deducting endogenous respiration values; oxidation of nonane = 100%. *x*: Individual hydrocarbons, denoted by number of carbons in molecule.

Oxidation of the various n-alkanes by *Candida lipolytica* 4-1 is illustrated in Fig. 1, which shows that pentane and hexane were not oxidized. The first signs of oxidation appeared in heptane. Nonane and decane were oxidized the most rapidly. With higher n-alkanes, the rate of oxidation fell as their molecular weight rose.

No differences were found in the rate of oxidation of synthetically prepared and natural n-alkanes isolated from gas oil, with the exception of undecane and tridecane. If prepared synthetically, these n-alkanes were oxidized much more rapidly than the same paraffins isolated from natural material, although their degree of purity was 99.9%. On removing the traces of the initial alcohols used for synthesis by further repurification, the oxidation values of both types of n--alkanes were the same.

Alkanes with a short side chain consisting of a methyl group were also oxidized, but at a significantly lower rate than the corresponding n-alkanes. The number of methyl groups influenced the oxidation rate; Qo_2 of alkanes with a single methyl group in the side chain was about 10 µl o_2 mg dry weight/ /60 min, while Qo_2 of alkanes with a methyl group at both ends was only half this value.

Table 2. Rate of oxidation of pure hydrocarbons by *Candida* lipolytica 4-1. Activity (Qo_2), after deducting endogenous respiration, is expressed in ml O_2 consumption/hour/mg yeast cell dry weight

Hydrocarbon	Qo_2	Hydrocarbon	Q_{O_2}
Pentane	0	Decene-1	43
Hexane	0.5	Undecene-1	76
Heptane	5.3	Tridecene-1	132
Octane	32	Tetradecene-1	29.7
Nonane	101	Hexadecene-1	32.6
Decane	94.4	Ostadecene-1	23.7
Undecane	76.8	Nonadecene-1	25.2
Dodecane	67	2-methyldodecane	10
Tridecane	49.3	3-methyltridecane	8.8
Tetradecane	46	2-methyltetradecane	92
Pentadecane	48.2	8-methylpentadecane	10.3
Hexadecane	46	2,11-dimethyldodecane	4.3
Heptadecane	43.8	2,13-dimethyltetradecane	6.5
Octadecane	41.6	Amylbenzene	7.3
Nonadecane	30.6	Hexylbenzene	23.2
Eicosane	30.6	Heptylbenzene	18.8
Heptene-1	3	Octylbenzene	28.4
Octene-1	0	Decylbenzene	25.8

The ability of *Candida lipolytica* 4-1 to oxidize hydrocarbons of the alkylaromatic series is very interesting. We tested the homologous series from ethylbenzene to decylbenzene. The first signs of oxidation appeared in amylbenzene.

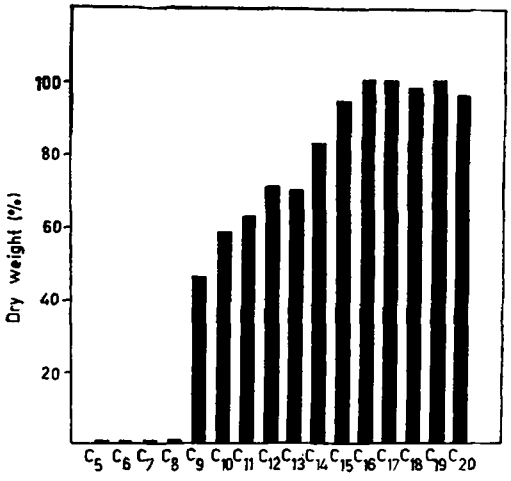

Fig. 2. Growth of *Candida lipolytica* 4-1 on pure n-alkanes. *y*: Biomass after 70 hours' cultivation, expressed in percentage form; growth on hexadecane = 100%. *x*: Individual hydrocarbons, denoted by number of carbons in molecule.

Alkylaromatic hydrocarbons with a side chain of less than five carbons in their molecule were toxic for the yeast cells, as seen from the decrease in endogenous respiration of a resting yeast cell suspension compared with the control. The benzene nucleus evidently has a positive effect on the oxidation of hydrocarbons with 5—7 carbons in the side chain. Pentane was not oxidized, while measurable oxygen consumption by *Candida lipolytica* cells was found in the presence of amylbenzene. Similarly, benzyl- and heptylbenezene were oxidized more rapidly than the corresponding n-alkanes. This effect was not manifested in alkylaromatic hydrocarbons with a longer side chain.

1-olefines were oxidized by *Candida lipolytica* more slowly than the corresponding n-alkanes, the only exception being tridecene, which was oxidized 2.5 times more rapidly than tridecane. Middle and high molecular weight 1-olefines were oxidized, analogical to n-alkanes. The first 1-olefine to be oxidized was 1-decene.

Table 2 shows the rate of oxidation of the individual hydrocarbons by *Candida lipolytica*, expressed as Qo_2.

Growth of *Candida lipolytica* 4-1 on hydrocarbons

Fig. 2 illustrates the growth of *Candida lipolytica* 4-1 on the various n-alkanes. The first n-alkane to be utilized was nonane, 45% of which — in relation to hexadecane — was converted to biomass. Biomass synthesis increased with the molecular weight of the n-alkanes up to hexadecane, over 85% of which was converted to biomass. Higher hydro-

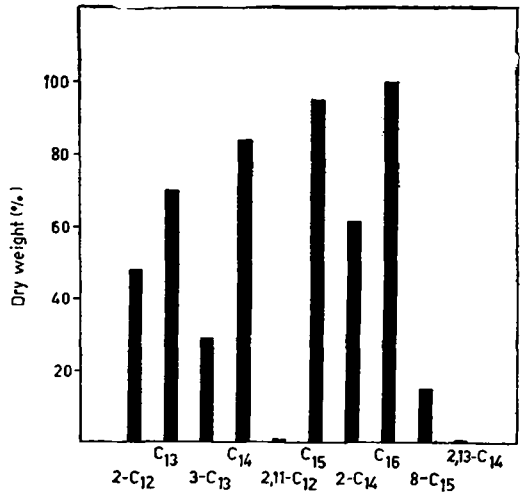

Fig. 3. Growth of *Candida lipolytica* 4-1 on isoalkanes. *y*: Biomass after 70 hours' cultivation, expressed in percentage form, biomass on hexadecane = 100%. *x*: Individual isoalkanes and n-alkanes, denoted by number of carbons in molecule. 2-C_{12} — 2-methyldodecane; 3-C_{13} — 3-methyltridecane; 2-C_{14} — 2-methyltetradecane; 8-C_{15} — 8-methylpentadecane; 2,11-C_{12} — 2,11-dimethyldodecane; 2,13-C_{14} — 2,13-dimethyltetradecane; C_{13} — n-tridecane; C_{14} — n-tetradecane; C_{15} — n-pentadecane; C_{16} — n-hexadecane.

carbons had practically the same conversion coefficient as hexadecane. Isoalkanes, which have a methyl side chain, were utilized for biomass synthesis less than the corresponding n-alkanes. The amount of biomass formed depends not

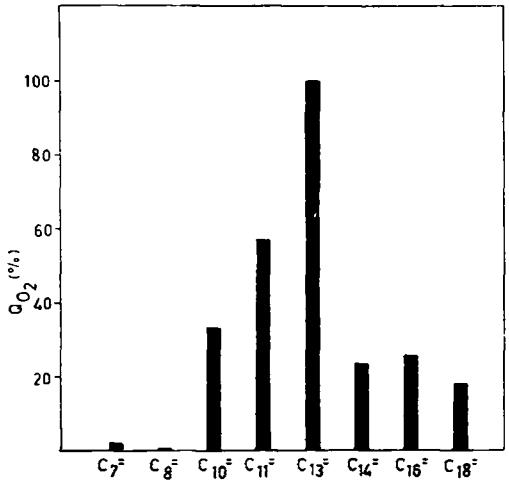

Fig. 4. Oxidation of pure 1-olefines by *Candida lipolytica* 4-1. *y*: Q_{O_2} values expressed in percentage form after deducing endogenous respiration values; oxidation of tridecane = 100%. *x*: Individual 1-olefines, denoted by number of carbons in molecule.

only on the size of the alkane molecule, but also on the site at which it branches. Isoalkanes whose chain branches at both ends (2,13-dimethyltetradecane, 2,11-dimethyldodecane) were not utilized at all, isoalkanes branched at one end (2--methyldodecane, 2-methyltetradecane) gave about 25—40% less biomass than the corresponding n-alkanes and the biomass yield on 8-methylpentadecane was only 20% compared with the amount of biomass formed on hexadecane, which has the same molecular weight. (Figs. 1, 2, 3.)

Although alkylaromatic hydrocarbons are oxidized, they are not utilized by *Candida lipolytica* 4-1 for growth. Among all the alkylaromatic substances tested, a minute increase in the amount of bio-

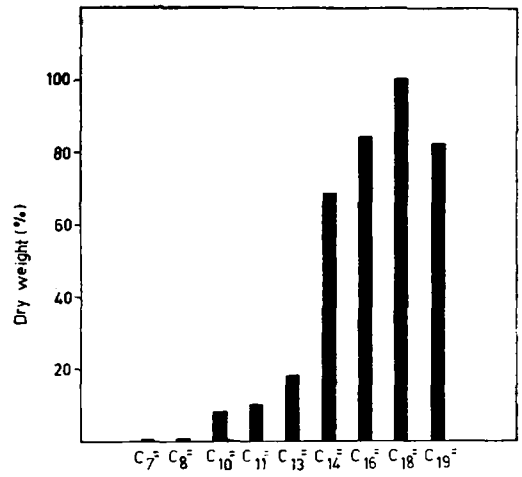

Fig. 5. Growth of *Candida lipolytica* 4-1 on pure 1-olefines. *y*: Biomass after 70 hours' cultivation, expressed in percentage form, growth on octadecane = 100%. *x*: Individual 1-olefines, denoted by number of carbons in molecule.

mass was found only when decylbenzene was used as the carbon substrate. This increase was only 5% compared with the yield obtained in cultivation on hexadecane.

The first 1-olefine capable of utilization was decene. As with n-alkanes, biomass synthesis rose with the molecular weight of the olefines. The maximum yield was obtained on octadecene. Comparison of the yields on 1-olefines with those on n-alkanes shows that the former are utilized less than the latter for biomass formation. With lower 1-olefines the difference amounted to as much as 85% and with higher 1-olefines to about 25%. The smallest difference — about 5% — was found in growth on octadecane or octadecene (Figs. 4, 5, 6).

Experiments investigating the rate of oxidation and the utilization of n-alkanes and 1-olefines by *Candida lipolytica* confirmed that the oxidation of hydrocarbons and their conversion to biomass were not directly correlated. The finding that hydrocarbons with a small molecule were oxidized rapidly, but were utilized

for cell synthesis less than middle and high molecular weight paraffins and 1-olefines is in agreement with the results of previous experiments (Dostálek *et al.*, 1968) in which we studied the successive degradation of n-alkanes in gas oil. The

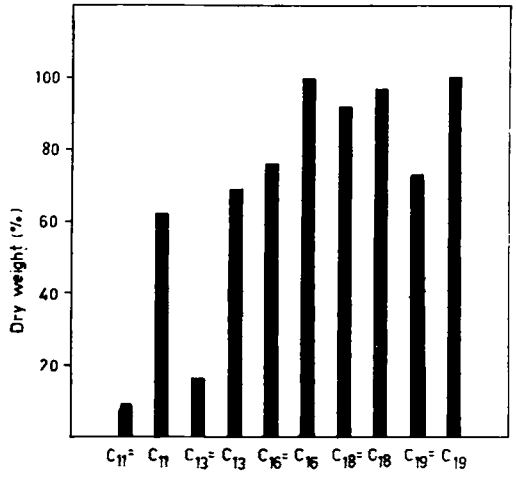

Fig. 6. Growth of *Candida lipolytica* 4-1 on n-alkanes and 1-olefines. *y*: Biomass after 70 hours' cultivation, expressed in percentage form; growth on hexadecane = 100%. *x*: Individual 1-olefines and n-alkanes, denoted by number of carbons in molecule. $C_{11}^{=}$ — undecene-1; $C_{13}^{=}$ — tridecene-1; $C_{16}^{=}$ — hexadecene-1; $C_{17}^{=}$ — octadecene-1; $C_{19}^{=}$ — nonadecene-1; C_{11} — undecane; C_{13} — tridecane; C_{16} — hexadecane; C_{18} — octadecane; C_{19} — nonadecane

occurrence of alkanes which were oxidized by the yeast, but on which it was unable to grow (heptane and octane), was very rare. In synthetically prepared alkanes, even minute impurity (0.1%) — usually by the initial alcohols — can lead to an increase in the rate of oxidation of the hydrocarbons. We observed this phenomenom with undecane and tridecane; the impurity is probably also the cause of the oxidative activity of a resting suspension of *Candida lipolytica* cells on 2,11-dimethyldodecane and 2,13-dimethyltetradecane, which are not be utilized for growth. Q_{O_2} of 2-methyltetradecane is likewise disproportionately high compared with other isoalkanes with a singel methyl group in the side chain; the rapid oxidation of 1-tridecene is probably also due to the presence of trace impurity by the initial alcohols.

The ability of yeast cells to utilize or to oxidize alkylaromatic hydrocarbons has not previously been described in the literature. The finding that these hydrocarbons, up to butylbenzene, act toxically on yeast cells (they inhibit respiration of a resting suspension) is in agreement with the known autolytic effects of these hydrocarbons.

The ability of *Candida lipolytica* to oxidize isoalkanes branched at one end of the chain and to oxidize higher alkylaromatic hydrocarbons indicates that alkanes are oxidized by the yeast to monocarbonic acids rather than to α-, ω dicarbonic acids. On the other hand, the lower rate of oxidation of isoalkanes and alkylaromatic hydrocarbons compared with the corresponding n-alkanes does not preclude the possibility of parallel oxidation to dicarbonic acids in the case of branched chain hydrocarbons.

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