J. Soc. Cosmet. Chem., **22**, 851–865 (December 9, 1971)

Assimilation of Selected Cosmetic Ingredients by Microorganisms

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Presented May 24–25, 1971, Seminar, Washington, D. C.

Synopsis—A study was made of the possible ASSIMILATION by various MICROORGANISMS of INGREDIENTS commonly used in the formulation of COSMETIC products. Hydrocarbons, high molecular weight alcohols, esters and fatty acids, and silicones were studied. Microorganisms used in this study were isolated from cosmetic products. Some species of PENICILLIUM, CANDIDA, and PSEUDOMONAS were noted to demonstrate strong ability to assimilate some of these ingredients. Of the materials tested, mineral oil, oleyl alcohol, stearyl alcohol, propylene glycol, isopropyl myristate, 2-hexyldecyl myristate, oleic acid, and stearic acid were found to be utilized as the sole source of carbon by most of the test organisms. Solid paraffin, multiwax, camellian, squalane, silicones, hexadecyl alcohol, polyethylene glycol, and di(2-hexyldecyl) adipate were not utilized by the organisms. The relationship between microbial assimilation and CHEMICAL STRUCTURE of substrate was also determined by the use of different esters.

INTRODUCTION

The ability to utilize hydrocarbons and their derivatives as the sole carbon source has been demonstrated by many different types of microorganisms. These organisms include fungi, yeasts, *Streptomyces, Nocardia,* and bacteria. Many of these organisms are found not only in the soil surrounding petroleum fields but in that of ordinary surroundings, including soil in which vegetables and fruits are grown.

It is well known that many different kinds of microorganisms are often isolated from cosmetic products. While some of these present no problems, others are responsible for changes in odor or breakdown of emulsion systems used in cosmetic formulation.

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Cosmetic products, in particular creams and lotions, are composed of an aqueous phase, an oil phase, and surfactants. Ingredients used in the formulation of the oil phase include hydrocarbons, high molecular weight alcohols, fatty acids, esters, silicones, and other substances.

Some of these ingredients were selected for this study and investigated in order to determine the microbial assimilation by organisms which were isolated from cosmetic products. Another objective of this investigation was to determine the relationship between assimilation and the chemical structure of selected substrates.

EXPERIMENTAL

Several different microorganisms were used in this study. They were isolated from various cosmetic products and included 23 strains of bacteria, 25 strains of yeasts, and 17 strains of fungi.

Preparation of Culture Media

Two different formulations of culture media were used. One medium was used for yeasts and fungi while another composition was used for bacteria. The compositions of the media are shown in Table I.

Various substrates were used in this study and represented the sole carbon source. Table II lists the materials which were used as the substrate.

Preparation of Organisms and Test Solutions

The bacteria and yeasts used in this study were incubated on a slant culture at 30°C for 48 hr. These cells were then suspended in a 0.006%

Table I
Compositions of Media

	For Bacteria (grams)	For Yeasts and Fungi (grams)
KH_2PO_4	0.5	2.3
K_2HPO_4	2.5	0.7
$MgSO_4$	0.3	0.3
$CaCl_2$	0.3	0.3
$(NH_4)_2SO_4$	3.0	3.0
NaCl	0.3	0.3
Water	1000	1000
Substrate	10.0	10.0
pH	6.8–7.0	5.5–5.6

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Table II
List of Tested Materials

Chemical	Materials[a]	Abbreviation
Hydro-carbons	Liquid paraffin-SHP 160	L.P.-1
	Liquid paraffin-Brandol	L.P.-2
	Vaseline	V
	Solid paraffin	S.P.
	Multiwax	M.W.
	Camellian[b]	Cam.
	Squalane	S.Q.
Silicones	Silicone KF-96[c]	KF-96
	Silicone KF-56[d]	KF-56
Alcohols	Oleyl alcohol	Ol Al
	Stearyl alcohol	St-Al
	Hexadecyl alcohol	HDA
	Propylene glycol	P.G.
	Polyethylene glycol 400	PEG-400
Esters	Isopropyl myristate	IPM
	2-Hexyldecyl myristate	E-103
	Diisopropyl adipate	IPA
	Di(2-hexyldecyl) adipate	E-201
Fatty acids	Oleic acid	Ol-Ac
	Stearic acid	St-Ac

[a] The commercial grade was used without further purification.
[b] Dimer dipentene manufactured by Japan Fine Chemicals, Koube, Japan.
[c] Dimethyl polysiloxane manufactured by Shinetsu Chemical Co. Ltd., Tokyo, Japan.
[d] Methyl phenyl polysiloxane manufactured by Shinetsu Chemical Co. Ltd., Tokyo, Japan.

Lapisol* solution and pipetted into several 100-ml Erlenmeyer flasks containing 30 ml of culture medium. The flasks, set onto a reciprocal shaker, were incubated for 10 days at 30°C. When larger quantities were necessary for the study, a 500-ml flask containing 150 ml of medium was used.

The fungi were treated in a somewhat different manner. Spores, which had been incubated at 25°C for 10 days and suspended in 0.006% Lapisol solution, were inoculated into 50-ml conical flasks containing 20 ml of media. The flasks were held stationary and incubated for 20 days at room temperature.

Determination of Growth of Organisms

In order to determine the growth of bacteria and yeasts, a cell suspension was prepared as indicated in Fig. 1. The optical density at 660 mμ

* Anionic surfactant manufactured by Nippon Oil & Fats Co., Ltd., Tokyo, Japan.

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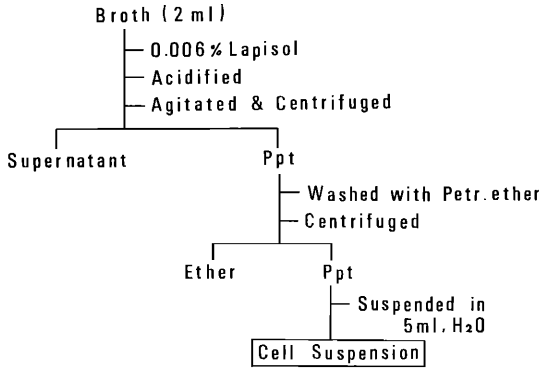

Figure 1. Preparative procedure of cell suspension

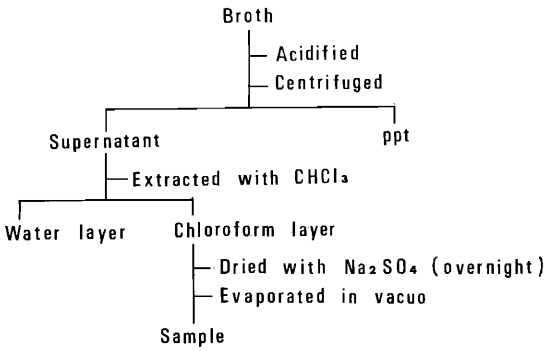

Figure 2. Extractive procedure of oil parts

was measured by a spectrophotometer for each of the cell suspensions. This result indicated the relative concentration of organism present before incubation. Following incubation, the broth was treated as shown in Fig. 2.

The conversion of substrates due to the organism was studied by gas-liquid chromatography and infrared absorption. A Varian Aerograph Series 1200 gas chromatograph was used. It was fitted with a 3 ft $\times$ $^1/_8$ in. column using Chromosorb AW (80/100) as the solid support. The stationary phase consisted of SE-30 (3%) and nitrogen at 30 ml/min was used as the carrier gas. The unit was fitted with a FID detector and was temperature programmed at 100°C with a rise of 8°C per minute.

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Table III

Microbial Assimilation of Materials by Bacteria[a]

	Gram-Positive Bacteria												Gram-Negative Bacteria										
	Bacillus							Unknown					*Pseudomonas*						Unknown				
	4	6	7	8	9	12	14	1	10	11	13	17	16	21	22	23	25	28	20	2	19	27	29
L.P.-1	−	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	−
L.P.-2	−	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−
V	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	−
S.P.	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−
M.W.	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	−	+	−	−	−	−
Cam.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
S.Q.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KF-96	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KF-56	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ol-Al	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	−	−	−	+
St-Al	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−
HDA	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P.G.	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	−
PEG-400	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
IPM	−	+	−	−	−	−	+	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	+
E-103	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+
IPA	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−
E-201	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−
Ol-Ac	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+
St-Ac	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	+

[a] Symbols: +, good growth; ±, slight growth; −, no growth.

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Table IV

Microbial Assimilation of Materials by Yeasts[a]

	Candida																			*Pichia*		Unknown			
	1	4	5	6	8	9	10	11	12	14	15	16	17	18	19	20	23	25	26	2	22	3	7	13	21
L.P.-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
L.P.-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
V	+	+	+	−	+	+	+	−	−	+	−	+	+	+	+	+	−	+	+	−	+	+	−	+	−
S.P.	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
M.W.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Cam.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
S.Q.	−	−	±	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KF-96	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KF-56	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ol-Al	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
St-Al	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−
HDA	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P.G.	+	−	+	−	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+
PEG-400	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
IPM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E-103	+	+	−	−	+	+	−	−	−	+	+	−	+	+	−	−	+	+	−	+	+	+	−	−	+
IPA	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	+
E-201	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ol-Ac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
St-Ac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+

[a] Symbols: +, good growth; ±, slight growth; −, no growth.

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Table V

Microbial Assimilation of Materials by Fungi[a]

	Tr.[b]	*Aspergillus*			*Penicillium*							*Cl.*[c]	Unknown				
	1	2	C	D	4	5	7	9	10	A	B	E	3	6	8	11	13
L.P.-1	−	±	−	+	+	±	+	+	+	+	+	±	−	+	+	±	±
L.P.-2	−	±	−	+	+	±	+	±	+	+	±	±	−	+	±	−	±
V	−	−	−	+	+	−	±	−	+	+	±	−	−	±	±	−	±
S.P.	−	−	−	±	+	−	−	−	−	±	±	−	−	−	−	−	−
M.W.	−	−	−	±	±	−	−	−	−	±	−	−	−	−	−	−	−
Cam.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
S.Q.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KF-96	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KF-56	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ol-Al	±	+	+	+	+	+	+	+	+	+	+	+	+	+	±	±	+
St-Al	±	±	±	+	±	±	+	−	±	+	±	±	−	+	±	−	+
HDA	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P.G.	+	−	−	−	+	+	+	±	−	−	−	+	+	−	±	+	+
PEG-400	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
IPM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E-103	+	+	+	+	+	±	+	+	+	+	+	+	+	+	±	+	+
IPA	−	±	−	−	+	−	+	+	−	±	+	−	−	±	−	−	−
E-201	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ol-Ac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
St-Ac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] Symbols: +, good growth; ±, slight growth; −, no growth.

[b] *Tricoderma.*

[c] *Cladosporium.*

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Discussion of Results

The results of the utilization of materials for growth by the various microorganisms are shown in Tables III–V. Table VI shows the assimilation rate of the substrates studied.

On the assumption that a material having an assimilation rate (AR) of over 0.5 can be easily utilized, it can be noted that about half of the tested materials were easily utilized by the organisms as the sole carbon source. These materials were liquid paraffins, oleyl alcohol, stearyl alcohol, propylene glycol, isopropyl myristate, 2-hexyldecyl adipate, oleic acid, and stearic acid. It can also be seen that materials with an AR close to zero were not utilized by the microorganisms. These were solid paraffin, multiwax, camellian, squalane, silicones, hexyldecyl alcohol, di(2-hexyldecyl) adipate, and polyethylene glycol.

Table VI
Number[a] of Organisms Utilizing Materials Tested

Substrates	Bacteria (23 Strains)	Yeasts (25 Strains)	Fungi (17 Strains)	AR[b]
L.P.-1	9	24	9 (4)	0.65
L.P.-2	8	24	6 (7)	0.59
V	6	17	4 (5)	0.42
S.P.	7	3	1 (2)	0.17
M.W.	5	0	(3)	0.08
Cam.	0	0	0	0
S.Q.	0	1 (1)	0	0.02
KF-96	0	0	0	0
KF-56	0	0	0	0
Ol-Al	7	25	14 (3)	0.74
St-Al	6	19	5 (9)	0.46
HDA	0		0	0
P.G.	8	18	8 (2)	0.52
PEG-400	0	0	0	0
IPM	12	25	17	0.83
E-103	8	14	15 (2)	0.57
IPA	6	6	4 (3)	0.25
E-201	(1)	0	0	0
Ol-Ac	8	25	17	0.77
St-Ac	10	24	17	0.78

[a] ( ) indicates strains showing slight growth.

[b] $AR = \frac{\text{Total of growing strains}}{\text{Total of strains tested}}$.

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As shown in Table III, the bacteria isolated from cosmetics and studied were mainly classified as the *Genus Bacillus* and *Pseudomonas*. Some species of *Pseudomonas* exhibited a strong affinity to assimilate these selected materials.

Most of the yeasts (Table IV) were classified into the *Genus Candida* and also demonstrated a strong affinity to assimilate these materials. Among the fungi (Table V), many species of *Penicillium* were found which demonstrated a strong ability to assimilate these materials. The assimilabilities of some genera of microorganisms are shown in Table VII.

The typical growth curves of yeasts in the medium containing LP-1 (Fig. 3) demonstrate that the adaptation of yeast against liquid paraffin is different among strains. Some of them had a short lag time and others a long one. Inducible enzymes appear to be operative in the utilization of liquid paraffin. Various types of liquid paraffin are used in cosmetic formulations. The utilization of representative liquid paraffins by *Candida SY-15* is shown in Table VIII.

This organism utilized the tested liquid paraffin fairly well but the cell growth became poor as the viscosity of liquid paraffin increased. It may be expected that highly viscous liquid paraffin is composed mainly of isoparaffins or naphthenes and it can be noted that *Candida SY-15* hardly utilized them. The infrared absorption spectrum of the extract is shown in Fig. 4. Absorption at 1710^{-1} cm indicates the presence of carboxyl group.

Figure 5 shows one of the results obtained by the GLC technique. The upper chromatogram is the initial composition of liquid paraffin (SHP-160), and the lower chromatogram shows the same liquid paraffin after incubation. The sharp peaks correspond to *n*-alkanes. The peak

Table VII
Assimilability of Some Genera

Class	Genus	Sum of Materials Utilized
Bacteria	*Bacillus*	2–4
	Pseudomonas	14–16
Yeast	*Pichia*	11
	Candida	12–16
Fungi	*Aspergillus*	8–13
	Penicillium	12–15
	Cladosporium	10

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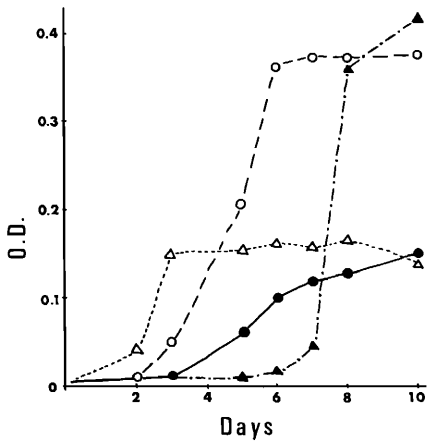

Figure 3. Growth curves of various yeasts in medium containing liquid paraffin (paraffin-rich)

Table VIII
Utilization by *Candida SY-15* of Several Liquid Paraffins Used as Substrates

Trade Name	Manufacturer	Composition	Viscosity (cps)	Growth[a]
SHP-160	Union Carbide Corp.	Paraffin-rich	8.4	++
Crystol-70	Esso Standard Oil Co. Ltd.	Paraffin-rich	13.6	+
Blandol	Witco Chemical Corp. Sonneborn Div.	Paraffin-rich	16.5	+
SHP-280	Union Carbide Corp.	Paraffin-naphthene	25.2	+
Drakeol-35	Pennsylvania Refining Co.	Naphthene-rich	74.0	±

[a] Symbols: ++, abundant growth; +, moderate growth; ±, slight growth.

height of *n*-alkanes (C_{14}–C_{19}) decreased sharply after incubation. It can be noted that *Candida SY-15* utilizes *n*-alkanes in liquid paraffin as the carbon source and converts a terminal methyl group to a carboxyl group.

The relationship between microbial assimilation and the chemical structure of substrate was studied using selected esters. The esters used as well as the results obtained with *Pseudomonas No. 23* are shown in Table IX. These esters consisted of straight-chain compounds and the basic group (myristic acid, adipic acid, or glycerol) was utilized easily by the test microorganism. However, in the case of branched chains which were bound to the basic compound, the utilization was poor as the ester

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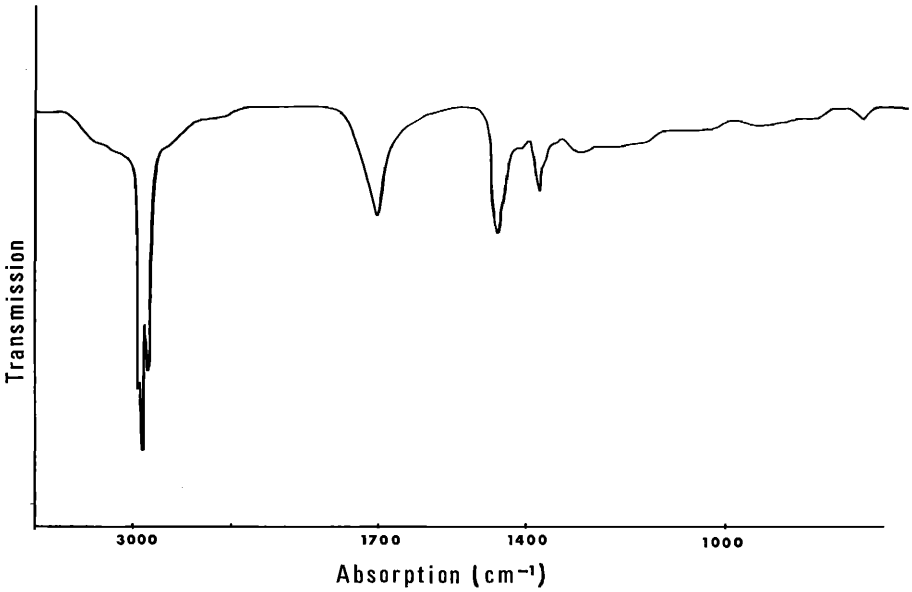

Figure 4. Infrared spectrum of liquid paraffin (paraffin-rich) after incubation

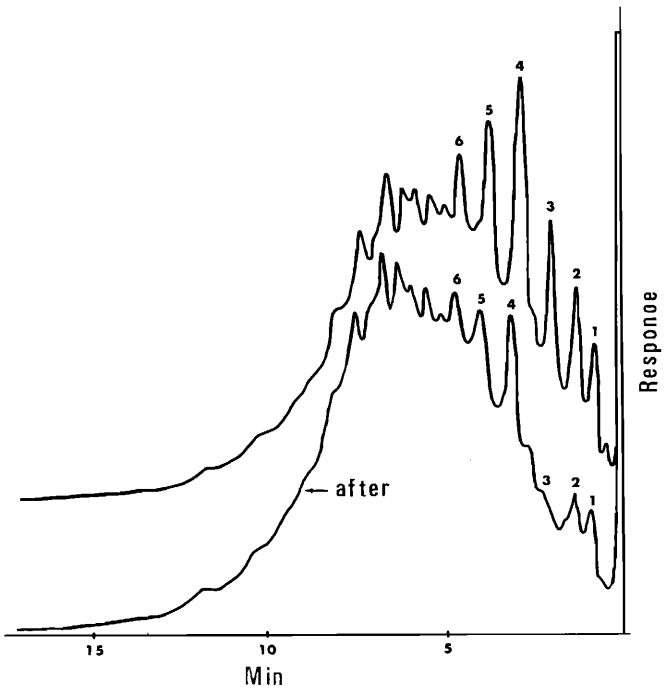

Figure 5. Chromatogram of liquid paraffin (paraffin-rich)

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Table IX

Assimilation by *Pseudomonas No. 23* of Various Esters

Ester	Structure	Growth[a]
Myristyl myristate	$CH_3(CH_2)_{12}COOCH_2(CH_2)_{12}CH_3$	++
Isopropyl myristate	$-CH(CH_3)_2$	++
2-Hexyldecyl myristate	$-CH_2CH(C_6H_{13})(C_8H_{17})$	+
Diisopropyl adipate	$[-CH_2CH_2COOCH(CH_3)_2]_2$	+
Di(2-ethylhexyl) adipate	$[-CH_2CH(C_2H_5)(C_5H_5)]_2$	±
Di(2-hexyldecyl) adipate	$[-CH_2CH(C_6H_{13})(C_8H_{17})]_2$	−
Glycerol tricaprylate	$CH_2OCOCH_2(CH_2)_5CH_3$ | $CH-O-$ | CH_2O-	++
Glycerol trimyristate	CH_2O- | $CH-OCOCH_2(CH_2)_{11}CH_3$ | CH_2O-	++
Glycerol tri-2-ethylhexanoate[b]	CH_2O- | $CH-OCOCH(C_2H_5)(C_4H_9)$ | CH_2O-	−

[a] Symbols: ++, abundant growth; +, moderate growth; ±, slight growth; −, no growth.

[b] Synthesized in laboratories of Shiseido Co.

group increased. In the case of diesters, the utilization became more difficult as the branched chain length was increased. The gas chromatograms shown in Figs. 6 and 7 illustrate the degradation of the glycerides. As for glycerol tri-2-ethylhexanoate, there was little difference between before and after incubation. In the case of glycerol tricaprylate, three new peaks appeared after incubation and the peak for triglyceride decreased. These three peaks are perhaps free fatty acid, monoglyceride, and diglyceride (right to left).

A time course of the biodegradation of glycerol tricaprylate is represented in Fig. 8. The content of triglyceride and the pH value of the

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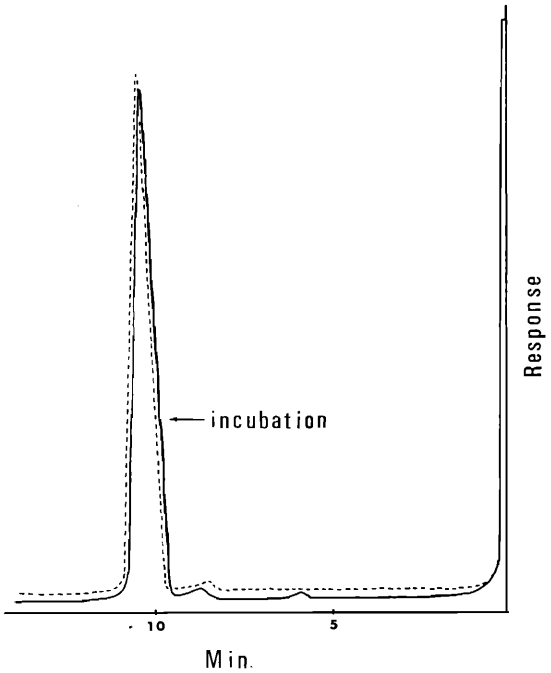

Figure 6. Chromatogram of glycerol tri-2-ethylhexanoate

medium decreased as the bacterial growth increased. Monoglyceride and diglyceride appeared as the triglyceride diminished but their content did not increase appreciably. It may be supposed that mono- and diglyceride were utilized as the sole carbon source.

Glycerides are thought to be nutritious to microorganisms. However, the glyceride synthesized with branched-chain fatty acid was not utilized. The branched chain demonstrated a great resistance against microbial assimilation. Similar results were noted with fatty acids and esters.

The biodegradability of branched-chain derivatives were studied with hydrocarbons (1–4) and alkyl benzene sulfonates (ABS) (5). As for aliphatic hydrocarbons, it was reported that *n*-alkanes or *n*-alkenes were readily assimilated and the assimilation became more difficult as side-chain length increased. With ABS, it was found that the compounds with the phenyl group nearer the center of the chain were much more resistant than others with 2- and 3-phenyl isomers. The methyl group in the chain did not retard the degradation and little difference was noted

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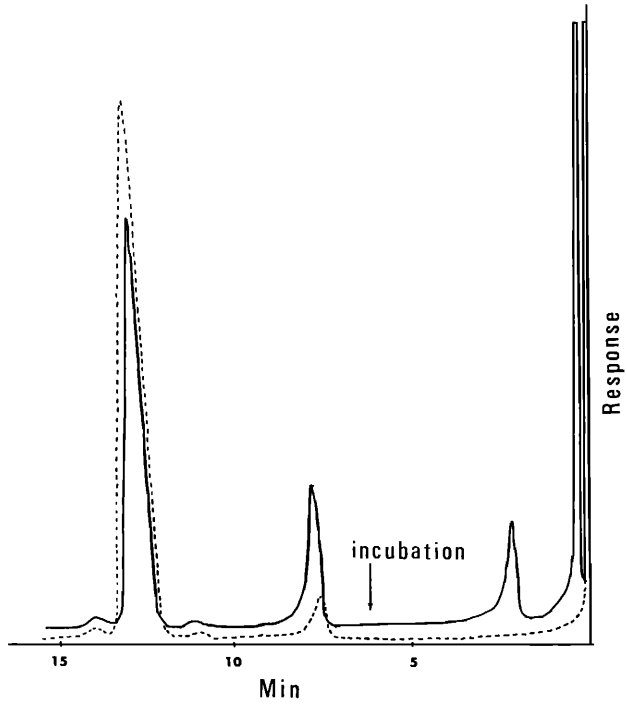

Figure 7. Chromatogram of glycerol tricaprylate

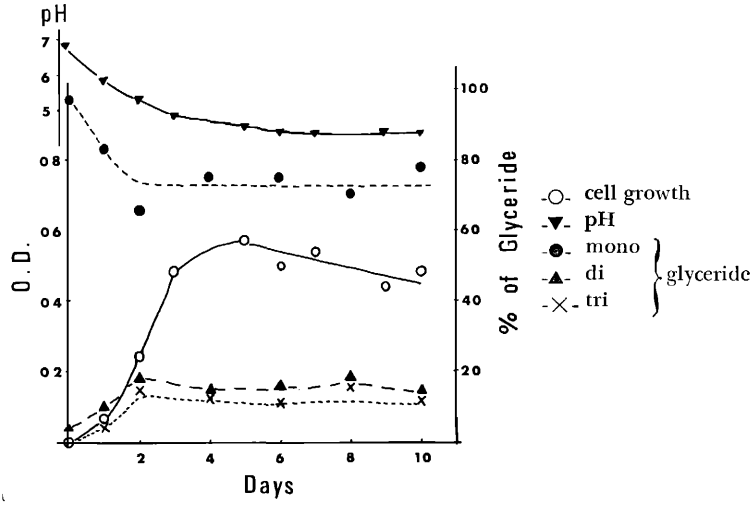

Figure 8. Degradation of glycerol tricaprylate

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whether it was at the near end of the chain or at the far end. For any given carbon number, the degradation was slower with increased branching.

As mentioned previously, this study was not carried out using compounds having the same carbon number, but by using branched-chain compounds and increasing the length of the branched chain so that the microbial assimilation could be retarded. Our conclusion is in agreement with the general findings on branched-chain derivatives utilization by microorganism.

Acknowledgment

The authors wish to express their sincere thanks to Professor M. Nakano, Faculty of Agriculture, Meiji University, for his useful suggestions during this study. They also wish to acknowledge the technical assistance of Mr. T. Sakamoto, Mrs. Y. Hirano, and Miss Y. Dobashi.

(Received May 26, 1971)

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