

# Natural Oils and Fats as Fermentation Sources\*

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醱酵源으로서의 天然油脂

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## Introduction

Will it be possible to resolve the problems of resources and energy in coming days as population increases? Even if we resolve the energy problem with atomic and solar energy after consuming petroleum energy completely, how can we get resources, especially food and feed?

As such there are limitations in the improvement of agricultural techniques, augmentation of cultivated acreage, and promotion of fisheries for solving these problems. We can propose two ways to approach these problems. The first is to increase the productivity of agricultural products by the methods of genetic engineering and related fields, and the second is to utilize the natural resources efficiently. The second is feasible and realistic in these days. Therefore, the growing concern has been recently focused on renewable resources such as starch and cellulosic materials as for fermentation. The problems would be solved if we could utilize the cellulosic substances effectively. Unfortunately we have not yet found the microorganisms which can degrade the cellulosic substances effectively like organisms degrading starch materials. It is not possible to anticipate finding such microorganisms, because of the structural properties of cellulosic substances.

Better utilization of fertilizers, improvement of genetic crosses, and tissue culture techniques have greatly increased the production of vegetable oils, especially rapeseed and palm

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\* A part of this work was published on *Yugakaku*, 33: 672-675(1984) in Japanese by Koh, J. S. and Y. Minoda.

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oil(Bruno andSchmid, 1982). The great increase of oil production has opened up subjects for research recently. Few reports, however, have been published on the utilization of natural oils and fats as the raw materials for fermentation by microorganisms. A survey of the literature indicates the studies on the microbial utilization of vegetable oils including the production of antibiotics, organic acids and biosurfactant. The addition of lipid materials enhances the yields in lipase production. Among the studies on microbial utilization of oils and fats, single cell protein productin has been carried out recently, especially from palm oil.

This review considers microbial utilization of natural oils and fats as raw materials for fermentation. Particular emphasis is given to single cell protein production from palm oil.

### Antibiotics Production

It is well known that the use of fatty oils, that is, animal and vegetable oils, accelerate the penicillin production. These oils have been shown to be readily metabolized by the fungus and serve as the main energy source for the fermentation. Anderson et al. (1956) investigated the effect of oil as antifoam agents in penicillin fermentatin on a pilot plant scale. Nearly all the added lard oil was catabolized by *Penicillium chrysogenum* Wis. 49-133 if the additions were kept at a level of 0.1%. The unsaturated fatty acids disappeared from the medium at a faster rate than the saturated acids. Pan et al. (1959) used fatty oils of high content of oleic or oleic plus linoleic acids as an energy source for penicillin fermentation, the fatty oils could be very efficiently utilized by the strain. With a proper ratio of mineral oil to fatty oil, such as corn oil, the fermentation could proceed at a rate 50% higher than the lactose control, yielding a final potency almost twice as high. Lipid including animal and vegetable oils were also used as replacements for the glucose in media for the cultivation of antibiotic-producing *Streptomyces griseus* cultures without reduction in streptomycin production (Perlman and Wagman, 1952).

Preliminary investigation by Anderson et al.(1958) on the effects of various adjuncts on carotene synthesis by mated cultures belonging to the family *Choanephoraceae* revealed that the addition of vegetable oil to the fermentation medium greatly enhanced carotene production. Brock (1956) noted that the addition of various oils and fatty acids to the medium stimulated the fermentative production of filipin by *Streptomyces filipinensis*. This antibiotic is polyene as also is carotene. The synthesis of carotene by mated strains of *Blakeslea trispora* was considerably enhanced by the addition of various natural oils and greases to the medium. Oils containing primarily oleic and linoleic acids were particularly effective in stimulating  $\beta$ -carotene production (Ciegler et al., 1959).

### Microbial Metabolite Production

Tabuchi et al. (1969) investigated the production of citric acid by *Candida lipolytica* No. 228 on fatty acids and natural oils. From materials such as glycerol, acetic acid, oleic acid, soybean oil, fish oil and linseed oil, citric acid was produced markedly in a yield of 30–50%. Ikeno et al. (1975) also carried out citric acid production from natural oils and fatty acids using a mutant strain of *Candida lipolytica* 281 obtained by mutagenic treatment with N-methyl-N'-Nitro-N-nitroguanidine. In particular, 102 g / ℓ of citric acid was produced from palm oil by shake culture, and the yield of citric acid against oil was 146%. *Candida tropicalis* was cultured aerobically on a medium containing palm oil to produce citric acid by Masuda et al. (1976).

It has been widely reported that the production of lipase by microorganisms increases in the presence of triglycerides and other lipids. Unsaturated long-chain fatty acids and vegetable oils such as oleic, linoleic, ricinoleic acid, and olive oil were effective on the lipase production by *Candida paraliipolytica*, but saturated fatty acids and coconut oil were not effective (Ota et al., 1968; Sugiura et al., 1975). The effect of lipid materials addition on the lipase production by *Torulopsis ernobii* (Yoshida et al., 1968), *Geotrichum candidum* (Tsujiisaka et al., 1973), and *Mucor hiemalis* (Akhtar et al., 1980 and 1983) has been reported. Addition of 1% triglycerides to the fermentation medium was best for the mycelial as well as broth lipase production. The added triglycerides seemed to be utilized through the formation of free fatty acids, and most of the triglycerides and their hydrolysis products were utilized towards the end of the growth phase (Akhtar et al., 1983).

Kawashima et al. (1983) investigated extracellular production of a mannosylerythritol lipid by a mutant of *Candida* sp. from vegetable oils. The strain produced the biosurfactant in concentrations of 27 to 35 g / ℓ of culture broth from triacylglycerols under suitable conditions, and yield amounted to 55 to 65% on a weight basis of carbon sources supplied. Cooper and Paddock (1984) also investigated biosurfactant production by *Torulopsis bombicola* ATCC 22214 from vegetable oils. A burst of surfactant production was induced when the yeast was grown on a glucose containing medium and then added vegetable oil after the exponential growth phase. The maximum yield of glycolipid was 70 g / ℓ, or 35% of the weight of the substrate used.

### Treatment of Waste Water Containing Fatty Oils

Barker et al. (1981) and Barker and Worgan (1981) investigated the utilization of palm oil processing effluents as substrates for microbial protein production by *Aspergillus oryzae*, and composition and nutritional evaluation of this fungus. Biomass yields of approximately 50g

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per 100g organic matter were obtained containing 40% crude protein, with BOD reduction of 85% and COD reductions of 75% to 80% in batch culture. This studies seem to be significant in the resolution of environmental pollutant. Green et al. (1976) investigated lipolytic fermentations of stickwater by *Geotrichum candidum* and *Candida lipolytica*. Stickwater, a by-product of the fish meal and oil industry, is an aqueous suspension of fish proteins, lipids, and other materials, and also contains soluble nonprotein nitrogen but no carbohydrate. The investigation was undertaken to digest and solubilize lipids in stickwater by lipolytic fermentation and to attempt to increase the protein content as single cell protein.

#### Single Cell Protein Production

The studies on the production of single cell protein (SCP) using a medium containing animal or vegetable oils and fats, especially palm oil, have made progress recently. SCP production was carried out with a substrate of animal fats (Vass et al., 1976; Kajs and Vanderzant, 1979), fish oil (Hottinger et al., 1974), soapstocks from palm oil (Ba et al., 1981; Martinet et al., 1982a and 1982b), rapeseed oil (Montet et al., 1983), olive oil (Tan and Gill, 1984), and palm oil (Koh et al., 1980; Koh, 1981; Nakahara et al., 1983; Koh et al., 1983, 1985a and 1985b). Most strains capable of assimilating oils and fats are lipolytic or hydrocarbon-assimilating organisms, and the strains could utilize unsaturated fatty acids more easily than saturated acids. Studies by Hottinger et al. (1974) probably comprise the first research on microbial utilization of triglycerides, especially fish oil, for cell production. Vass et al. (1976) cultivated *Candida utilis* in a cultured medium containing 1% pork fat. The proliferation was carried out at 30°C and pH 4.5 to 5.0. After 12 hrs, the fat was completely consumed, and cell yield was 75%. The protein content of the cells was at least 50%, and yeast cells were rich in B-vitamins. Kajs and Vanderzant (1979) also investigated utilization of tallow by food yeasts, *Sacchromycopsis lipolytica* and *Candida utilis*. The type and percentage of fatty acids utilized during the growth of the strains were examined by GLC. The mechanism of utilization of tallow by the yeast cells was supposed to be the cellular resynthesis of the oxidized, degraded fatty acids, and a chain elongation system which can add one or more C<sub>2</sub>= units to fatty acids. Ba et al. (1981) screened yeast strains from stock cultures for cell production. The specific growth rate and cell yield of *Candida lipolytica* YB 423-12 and *C. tropicalis* CBS 6320 were 0.27, 0.23 hr<sup>-1</sup> and 60, 72%, respectively. Martinet et al. (1982) also investigated growth parameters on soapstocks and palm oil stearin. The strain of *C. lipolytica* YB 423-12 grew well over the range of pH 3.5 to 7.5, and cell yield on palm oil stearin was 1.48g of cell per g of substrate. However, when I calculate the cell

yield theoretically on the basis of substrate carbon, the yield is questionable. *C. blankii* CBS 1898 was chosen as the best strain on palm oil among those tested by Nakahara et al. (1982). Optimum temperature of this strain was 40°C. The cell yield and the specific growth rate in a shake culture were about 80% and 0.37 hr<sup>-1</sup>, and the crude protein content was about 40%. rapeseed oil and solid fraction of palm oil were used as the substrates for cell production (Montet et al., 1983). According to the growth rate, the best strains on rapeseed oil were *Candida rugosa*, *C. deformans*, *C. lipolytica* and *Geotrichum candidum*. On the solid fraction of palm oil the best strain among those tested was *C. rugosa* CBS 613. Growth yield was 0.8g of cell per g of substrate and protein content of the cells was 35% on average. Tan and Gill (1984) cultivated *Sacharomycopsis lipolytica* on olive oil, cell composition during exponential growth was 42% protein and 2% fat. As fermentations progressed, free glycerol appeared and concentrations of di- and monoglycerides passed through maximal values although peak concentrations of di- and monoglycerides persisted for extended times in oxygen- and nitrogen-limited cultures respectively.

Authors (Koh et al., 1983) isolated a yeast strain, *Torulopsis candida* Y-128, from soil source, and investigated cultural conditions for cell production from crude palm oil. When the substrate was emulsified with a nonionic surfactant, sucrose ester of fatty acid P-1670, yeast cell growth was promoted. The protein content of dried cells was over 40% and amino acids of the yeast protein were well-balanced. Optimum cultural conditions of this strain were 35°C and pH 3.5, which is more acidic than other yeasts. The specific growth rate and cell yield were 0.43 hr<sup>-1</sup> and 0.92 g of cell per g of substrate consumed, respectively in jar fermentor culture (Koh et al., 1985a). In the case of refined palm oil as the substrate, there was no significant effect on surfactant addition. This strain also could assimilate unsaturated fatty acids more easily than saturated acids. As shown in Table 1, oleic acid content in the residual palm oil decreased from 42.6 to 34.4% of the total fatty acids while palmitic acid increased from 39.9 to 48.1% during cultivation of *T. candida* Y-128 in a jar fermentor in stationary phase. *T. candida* JNUA 35, isolated from field soil of Cheju island, is supposed to have the same potency in cell production on crude palm oil (Koh, 1981).

Authors (Koh et al., 1985b) also isolated a bacterium, *Acinetobacter calcoaceticus* KB-2, capable of assimilating palm oil effectively from soil source. This organism grew with a maximum specific growth rate of 1.10 hr<sup>-1</sup>. The optimum temperature was 39 °C, which was thermophilic compared with other strains of *Acinetobacter* sp. The productivity of cell mass was high in a short cultivation time, and the overall yield was 1.02 g of cell per g of palm oil consumed after 8 hrs cultivation at a concentration of 3% palm oil (Fig. 1). The protein content of cells was 56.9% (crude protein, 72%) and amino acids of bacterial protein were well-

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Table 1. Fatty Acid Compositions of Palm Oil and Residual Oil from Refined Palm Oil during cultivation<sup>a</sup>

Compound	Crude palm oil	Refined palm oil	Cultivation time <sup>b</sup>	
			24 hr	24 hr
C 12:0	0.2	0.3	0.4	0.2
C 14:0	1.0	1.0	1.0	0.8
C 16:0	45.7	39.9	44.5	48.1
C 16:1	0.1	0.1	0.1	—
C 18:0	4.5	4.8	5.4	5.6
C 18:1	38.5	42.6	37.4	34.4
C 18:2	9.7	11.0	10.9	10.7
C 18:3	0.3	0.3	0.3	0.2

a. Crude palm oil was supplied by Kao Soap Co. Ltd. and refined oil was commercial (Nipon Oil and Fats Co. Ltd.), Fatty acid composition is expressed in terms of percentage of total fatty acids.

b. Fermentation was at pH 3.5 and 35°C in a jar fermentor culture.

balanced compared with FAO/WHO references (Table 2 and 3). The oxygen demand of this strain in palm oil fermentation was almost the same as that in hydrocarbon fermentation. When no product of any byproducts was assumed, the following mass balance was derived on elementary analysis of the cell mass and palm oil;

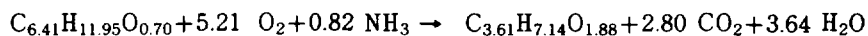


Table 4 summarizes the single cell protein production from oils and fats until recently. Studies carried out by the authors probably comprise the first comprehensive and promising research on microbial utilization of palm oil for cell production.

Table 2. Chemical Compositions of Yeast and Bacterial Cells (%)

	<i>Torulopsis candida</i> Y-128	<i>Acinetobacter calcoaceticus</i> KB-2
Protein(Lowry)	42.2	56.9
Crude nucleic acid	5.0	15.2
Total carbohydrate	40.3	9.5
Crude fat	3.4	10.0
Ash	6.8	7.9

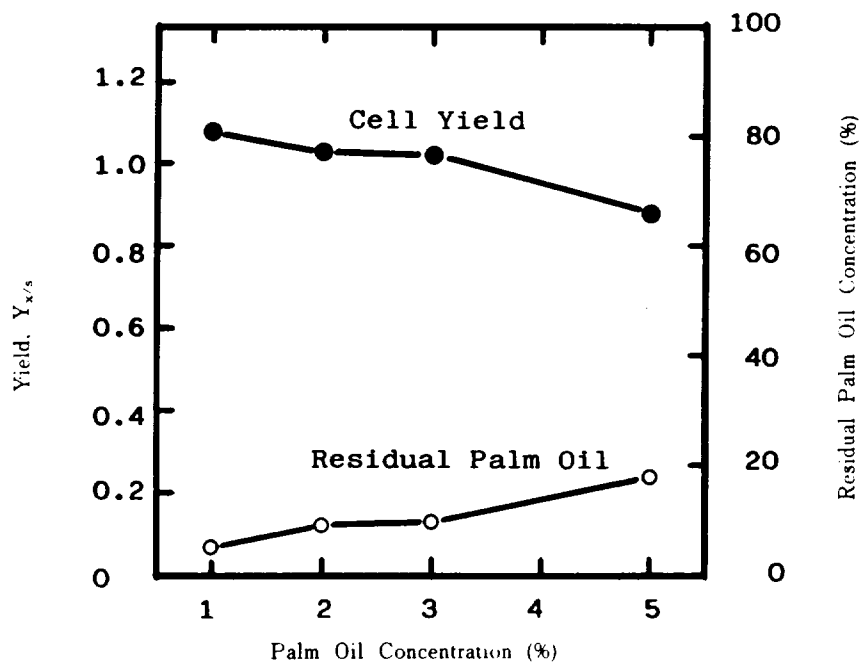


Fig. 1. Yield (●) and Residual Palm Oil Concentration (○) as a Function of Palm Oil Concentration in *Acinetobacter calcoaceticus* KB-2.

Table 3. Amino Acid Compositions of Microbial Protein (g/100g protein)

Amino acid	FAO/WHO reference	<i>T. candida</i> Y-128	<i>A. calcoaceticus</i> KB-2
Aspartic acid		8.33	11.28
Threonine	4.0	4.69	5.65
Serine		5.03	3.86
Glutamic acid		9.20	13.15
Proline		2.86	3.47
Glycine		3.86	5.40
Alanine		4.96	7.95
Valine	5.0	4.86	7.03
Cystine/2		1.61	0.89
Methionine	3.5 (Met+Cys)	2.56	2.86
Isoleucine	4.0	4.25	5.55
Leucine	7.0	6.42	8.89
Tyrosine		3.47	4.80
Phenylalanine	6.0 (Tyr+Phe)	4.21	4.38
Lysine	5.5	5.99	6.84
Histidine		1.95	2.10
Arginine		3.91	5.88

Table 4. single Cell Protein Production from Oils and Fats

Substrate	Microorganism	Cell yield*	Crude protein content	Specific growth rate	Reference
Tallow	<i>Candida utilis</i>	0.75	50 %	- hr <sup>-1</sup>	Vasset al.(1976)
Fish oil	<i>C. lipolytica</i>	0.81	46.8-49.3	0.24	Hottinger et al(1974)
	<i>Geotrichum candidum</i>	0.74	40.1-44.2	0.24	"
Fatty acids (Palm oil)	<i>C. lipolytica</i>	0.60	33	0.27	Ba et al.(1981)
	<i>C. tropicalis</i>	0.72	40	0.23	"
Rapeseed oil	<i>C. rugosa</i>	0.75	30	0.46	Montet et al.(1983)
	<i>C. deformans</i>	0.75	30	0.28	"
olive oil	<i>Saccharomycopsis lipolytica</i>	1.00	42	-	Tan and Gill(1984)
Palm oil stearin	<i>C. rugosa</i>	0.65	26	0.10	Montet et al.(1983)
Palm oil	<i>C. blankii</i>	0.78	40	0.37	Nakahara et al.(1982)
	<i>Torulopsis candida</i>	0.92	45.4	0.43	Koh et al.(1983,1985a)
	<i>Acinetobacter calcoaceticus</i>	1.02	72.4	1.10	" (1985b)

\* Cell yield is expressed as g cell/g substrate.



## Present Status and Future Developments

Nevertheless the growing concern has been focused on microbial utilization of oils and fats, few reports have been published on this subject except SCP production from palm oil. Natural oils and fats were used as a supplementary substrate in most works on the production of microbial metabolites, and the studies on cell production are only beginning. Is it due to the cost of oils and fats until recently, or from ineffectiveness of oils and fats as raw materials for fermentation?

Considering the price (Richtler and Knault, 1984) and high productivity of oils, especially palm oil, compared with other agricultural crops, the production of microbial cells or metabolites from oils and fats will be significant in supplying renewable resources for fermentation if oils are replaced by hydrocarbons. The serial works carried out by authors began to search for one of the possibilities on these points, and remarkable results were obtained recently.

The production of palm oil has recently been increased, and the World Bank estimated that palm oil production would increase to 11 million metric tons by the year 2000 (Mason and Ginar, 1980), although most palm oil is produced in Malaysia and should be exported, oil consumptions for food uses in the importing countries would not be expected to increase greatly in the near future. Therefore, it has been necessary to search for other uses of palm oil as same as other oils and fats. The studies on the utilization of oils and fats such as fuels for diesel engines (Harwood, 1984), raw materials for oleochemical and fermentation industries have been carried out. However, there arise numerous difficulties derived from the nature of oils and fats, such as chemical compositions, insolubility for substrate, softening point around cultivation temperature, and other biophysical properties as raw materials of fermentation, compared with other substrates.

In spite of the difficulties, it is known that dicarboxylic acids, organic acid and biosurfactant are produced industrially from palm oil and fatty oils recently by Japanese companies (personal communication). Continuous culture of *Acinetobacter calcoaceticus* KB-2 on palm oil, and cell production from vegetable oils such as rapeseed oil and soybean oil were also carried out by authors (unpublished data).

The studies on the microbial utilization of natural oils and fats are only beginning now, and further studies will be continued by many researchers in the future. This review may be attribute to search one of the possibilities in this points.

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## 國 文 抄 錄

油脂作物은 他作物에 비해 生産性이 매우 높으며, 最近 油脂生産量의 급격한 증가추세로 油脂 資源을 食用이외의 有效利用方案에 대한 관심이 증대되고 있는 實情이다. 따라서 微生物에 의한 油脂의 有效利用에 관한 1950年代에서 最近까지의 發表된 研究報告들을 分類整理하였다.

微生物醱酵源으로서 油脂는 항생물질, 有機酸, 酵素, 界面活性物質 등의 生産에 活用되어 왔 으며, 最近 油脂를 함유하는 工業廢水의 微生物處理 및 微生物에 의한 palm oil의 有效利用에 관 한 著者등의 研究結果를 중심으로 한 油脂에서의 微生物蛋白質生産 등 醱酵源으로서의 天然油脂 의 利用에 관한 現狀과 장래의 利用可能性 등을 검토하였다.