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Biotechnology and the Fats and Oils Industry – An Overview[1]

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ABSTRACT

Biotechnology is the application of single or multicellular organisms and of associated or derived enzyme systems to the production of desirable products. Particular discussion has been made of the derivation of fats and oils from animals, plants and microorganisms. General consideration has been given to methods, primarily plant breeding and agronomic practices for the improvement of the quantity and quality of oil produced by soybean, rapeseed, palm and sunflower. The possible importance of yeasts, fungi and algae as sources of single cell oil has been examined. A particular role of these systems in the production of specialty oils has been suggested. Enzyme systems, either associated with the intact cell or in isolation, can be used to varying degrees of success in either a free or immobilized form. Particular reference has been made to application of these systems to reactions including specific hydrolysis of triacylglycerols, acylation of glycerol, interesterification of triacylglycerols, wax ester formation and steroid transformations. Consideration has been given to particular plants and microorganisms as sources of new fats and oils. The major impact of biotechnology on the industry is believed to be associated with the production of high value specialty products including cocoa butter substitutes, biosurfactants, waxes and various prostaglandin derivatives. General consideration has been given to the possible relative importance of plant and microbial systems, engineering and scale-up problems, and overall economics of present biotechnological procedures.

INTRODUCTION

Definitions and concepts of biotechnology are many and varied, and reflect the particular view of the specialist. Thus biotechnology has been considered to be "the integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological application of the capacities of microbes and cultured tissue cells." Such a definition consequently excludes agriculture and medical technology, and emphasizes the application of microorganisms (1). A broader concept of biotechnology is based upon the unique characteristics of biological materials such as microbial, plant or animal cells and enzymes, and is the utilization of them or their components to provide goods or services (2). Although biotechnology is generally considered to be a new technology, many of its practices are based upon old technology. The modern biotechnologist simply builds on the old technology with newer ideas and techniques. The impact of the new biotechnology on the production of a wide variety of consumer goods—foods, pharmaceuticals, renewable fuel sources, chemical feedstocks, etc.—has yet to be fully realized. Potential applications of the new biotechnology in the area of fats, oils and derivatives are summarized in Figure 1.

Different fats and oils have been used by man since earliest times for a variety of purposes. The need for greater and better production initially required improvements in animal husbandry, agronomic practices and fermentation procedures. These changes mainly evolved without any profound scientific consideration until more recent times. Considered improvements in these general areas of old technology are continuing and are necessary adjuncts to the

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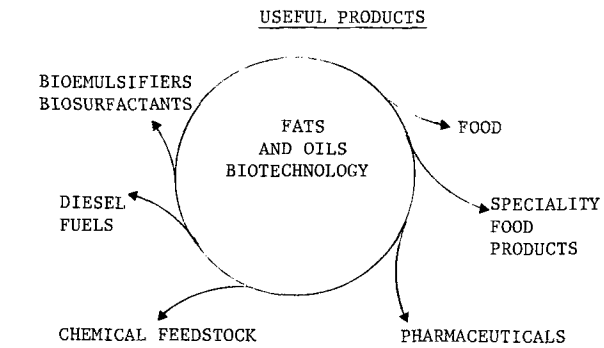

FIG. 1. Potential applications of biotechnology in the fats and oils industry.

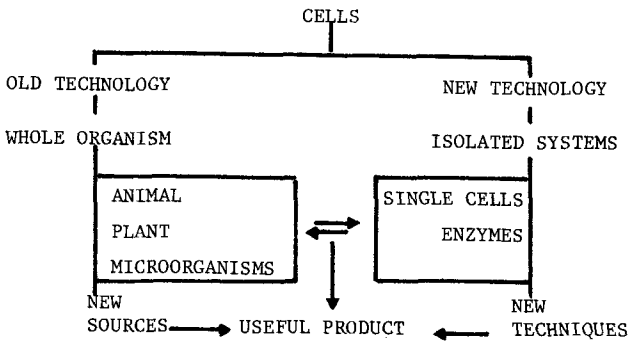

FIG. 2. Interrelationships between the old and the new practices of biotechnology.

newer practices of modern biotechnology. The old technology generally employs the whole organism, whether animal, plant or microorganism. Applications of single cells from animals and plants, as well as microorganisms, and the use of isolated enzyme systems, are associated with the new technology (see Fig. 2). Particular advances in biotechnology involving specific cell selection, growth practices, genetic engineering and enzyme applications can be anticipated. Thus biotechnology in the forms of old and new technologies is now available. Its particular relevance to the fats and oils industry is the timely topic for consideration.

PARTICULAR FATS AND OILS

Animal Fats

Dairy fats, and generally inedible lard, tallow and animal greases, are projected to constitute a smaller proportion of the total annual production of oils and fats at the beginning of the 21st century (3). Nonetheless, the actual needs have been projected to increase by approximately 40% above current production. Increased dairy fat can be predicted from selected cattle breeding and improved animal husbandry. It has been suggested that the increased demands for tallow could be met from growing genetically obese rats on (low cost) garbage (4). Fish oils, either for human nutrition or industrial purposes, will continue to be obtained by

TABLE I

The Oil Content and Areas of Desired Improvement for Four Major Oil-Bearing Plants

Crop	Oil content[a] (wt, %)	Areas of desired improvement	
		Oil	Plant
Soybean	18-20	Increased productivity.[b] Decrease in linolenic acid. Regulation of oleic acid desaturation and triacylglycerol synthesis.	Local adapted varieties. Photo-period, high-yielding cultivars. Virus and pest resistance.
Rapeseed	40-45	Decrease in linolenic acid.[c] Increase in linoleic acid.[c] Increase in erucic acid.[d] Decrease in chlorophyll and glucosinolate contamination.	Winter varieties. Disease and triazine resistance.
Palm	20	Increased productivity. Increase in unsaturation. Novel oil development.	Local adapted varieties. Crop management disease resistance. Decrease in biodeterioration of mature fruit.
Sun	40-45	Increased productivity. Increase in oleic acid. Decrease in wax.	Shorter varieties. Broader maturity range. Greater degree of self-compatibility.

[a]Reference 6.
[b]Not to be obtained at the expense of decrease in protein.
[c]LEAR.
[d]HEAR.

conventional methods. The practice of aquaculture, however, may assume greater significance and will involve selective fish breeding. It is possible that certain fish may be specifically reared for their oil content. A market has not yet been established for the significant quantity of oil by-product obtained during the processing of farm-raised catfish (5).

Plant Fats and Oils

The bulk of fats and oils, whether for human consumption or industrial usage, presently is derived from plant sources (6). Nonetheless, definite requirements exist for greater yields, better qualities and products of highly specialized chemical composition. Thus improvements are being made, not only with conventional crops, but also with selected plant species which have an ability to produce unique, desirable fats and oils (7). Some areas of desired upgrading of 4 major oil-producing plants are given in Table I.

Improvements in the quantity and quality of plant seed oils have been associated primarily with agronomic practices and plant breeding programs. Development of improved varieties was based intially on trial and error, but more recently on crossing, polyploidy or mutations (8). Application of the newer technology of tissue culture has tremendous potential for crop improvement and the development of unique germplasm (9). Methods for in vitro selection of such agriculturally valuable traits as disease resistance and stress tolerance have been found useful but limited in their applicability to certain species (10).

Manipulation of crop plants by genetic engineering so far has been limited (11,12). Use of the general procedures of genetic transformation and somatic hybridization shows promise (13), but will complement rather than replace conventional breeding procedures. Protoplast fusion methods can permit gene transfer between plants which cannot be crossed sexually (14). Thus, more effective resistance of plants to disease may be achieved. Despite the high potential of genetic engineering to crop improvement, the desired result frequently may be complicated by a variety of responses of plants to different environmental stresses (15).

Biotransformations by plant cell cultures have been considered for the production of secondary metabolites including steroids and terpenoids (16). Isolated suspension cultures or callus cultures of many plant species have proved useful in basic and applied research particularly on fats, oils and allied compounds (17). Further developments in the applications of immobilized plant cells can be fully anticipated (18-20). Large-scale production of shikonin in Japan and biotransformation of low value digitoxin to the important high value cardiatonic digoxin are now industrial realities (21).

Consideration has been given to the problems, both economic and engineering, associated with industrial scale-up of plant culture systems (22,23).

Soybean oil. Soybean oil continues to be the major edible oil used in the U.S. and throughout the world (3,6). The mature soybean has approximately 20% oil (24). In addition to improved yields, problems still exist with the oil quality (24). A particular factor is the relatively high content of linolenic acid (5-18% of the total fatty acid component), which has been associated with the oil's poor flavor stability and the generation of room odors. Several biotechnological approaches have been made in an attempt to solve this problem without having to resort to the cost-intensive industrial practice of hydrogenation of the linolenic acid. Application of recurrent selection methodology has resulted in new soybean genotypes which possess reduced contents of both linolenic and linoleic acids (25). Variations in the polyunsaturated fatty acid component, however, depend on the period (25) and ambient temperature (26) of seed development. Increased oil production with lower linolenic acid content occurs under warmer conditions (26). A com-

plex situation regulating soybean linolenic acid content has been envisioned as involving, among other factors, altered desaturase activity in the formation of linolenic acid and several systems for triacylglycerol biosynthesis (25). Linolenic acid levels in soybeans are believed to involve at least 4 or 5 different genes (24,27). The possibility of undertaking interspecific hybridization between cultivated soybean *Glycine max* (L) Merr. and species in the genus *Glycine* subgenus *glycine* would appear to hold little promise for the production of a low linolenic acid soybean (28).

Genetic manipulation to change soybean oil composition has been attempted through the use of mutagens such as ethylmethane sulfonate (29-31) in conjunction with conventional hybridization and selection procedures. Attempts to obtain germplasm possessing 3% or less linolenic acid appears to be approaching success. Decreased contents of linolenic acid also may be achieved through application to the soybean plant of certain substituted pyridazinones (32). While germplasm having a low linolenic acid value is sought, consideration also must be given to the retention of the nutritionally desirable linoleic acid content and to the maintenance and development of favorable agricultural traits.

Rapeseed oils. Although rapeseed oils have been used from earliest recorded times, quality improvements continue to be sought (33). Two major types of oils are recognized, LEAR (low erucic acid rapeseed) for edible use, and HEAR (high erucic acid rapeseed) for industrial applications. Although rapeseed has a generally high oil content of approximately 40% (33), attempts have been made both to decrease and increase the level of the erucic acid component. Systematic breeding programs of both rape *(Brassica napus)* and turnip rape *(B. campestris)* permitted development of cultivars with "zero" erucic acid content (34). A very low frequency of genes appears to be associated with the absence of erucic acid in rapeseed. The recent development of leaf mustard *(B. juncea)* genotypes low in erucic acid (35) should allow a wider market for the oil produced in China and India. Canadian-produced LEAR oil, designated as canola oil, contains $<5\%$ erucic acid as a percentage of the total fatty acids in the oil and <30 μmoles of glucosinolates per gram in the oil-free meal, and is now a major commercial oil (33).

The absence of erucic acid is generally accompanied by a reduction in eicosenoic acid content from 10% to approximately 1.5% of the total fatty acid component, while a major increase in oleic acid from approximately 15% to 60% occurs (34). "Zero" erucic acid oil also is characterized by increased levels of nutritionally-desirable linoleic acid and unstable linolenic acid. Values of linoleic acid approaching at least 30% of the fatty acid component appear to be genetically feasible (34). Development of genotypes giving higher linoleic acid-containing oils would be desirable. Chemically-induced monogenic mutants producing 35% linoleic acid and only 3% linolenic acid have been obtained (36). The oil yield and performance of these plants are generally poorer compared with the original cultivar. Further modification of the linolenic acid content will be complicated by the complex genetics involved (34). It is also difficult to separate genetic from environmental effects. The level of seed oil linolenic acid is increased when maturing rape plants are exposed to low temperatures and low light intensities (37,38).

Production of HEAR as a source of oleochemicals has been considered (39). Contents of $>50\%$ of the fatty acid component of the oil are regarded as desirable, but values of $>65\%$ have not been reported (34,40-42). Interest still exists in the possible genetic manipulation of rape to produce HEAR oil with a content of 80-90% erucic acid of the total fatty acid component (39). This will not be achieved until the inability of rape to incorporate erucic acid at position 2 of the glycerol moiety is overcome. Gene transfer from those plants, e.g. nasturtium *(Tropaeolum majus)*, which possess such activity, may be contemplated (43).

In all breeding programs of rape, consideration must always be given to achieving low levels of glucosinolates in the oil-free meal to allow its subsequent use as animal feed (34).

Palm oil. Although palm is a high oil-producing plant, the seed mesocarp has a relatively low oil content of 20% (6). Improved oil production has been sought through plant breeding and selection. More recently, tissue culture techniques have been used to select unique individuals for clonal propogation. In particular, certain clones of *Elaeis guineensis* have allowed the production of reproducible higher yields of palm oil of varying fatty acid composition (44). Hybridization of *E. guineensis* and *E. oleifera* has permitted the combination of the more desirable traits of both oil palm species (45). Based on theoretical considerations of the genetics of the process, predictions may be made regarding the triacylglycerol composition of mesocarp oils from different hybrid palms (45). It has been further suggested that certain clones of hybridization of *E. guineensis* and *E. oleifera* will permit introduction of plants not only producing a desirable more liquid oil but also showing greater disease resistance and easier crop management (44). Although the fatty acid composition of the mesocarp oil is under genetic controls, the extent of environmental factors influencing fatty acid composition has not been established. Only limited attention has been given to improving the kernel oil which is a valued commodity due to its high content of lauric acid (46).

Sun(flower) oil. The worldwide production of sun oil increased considerably with the availability of high oil varieties of *Helianthus annus* L. from the Soviet Union in the mid 1960s. Further advances in plant breeding and varietal improvement involved recognition of cytoplasmic male sterility and genes for fertility restoration (47,48). The average seed oil content is of the order of 40-45%. Certain hybrid plants in test plots have shown a capacity to produce 5600 kg oil/hectare compared with average present yields of 1200-1400 kg oil/hectare (48). Work is continuing on the development of seeds with higher oil content. Experimental lines having 63% oil in seed have been developed but are difficult to harvest, due to extremely thin seed hulls (48).

Sun oil has a nutritionally-desirable high content of linoleic acid which is sensitive to growth temperature ranging from 31-76% (49). Certain developed sunflower lines having an oleic acid seed oil content of 80-90% appear to be relatively temperature insensitive (48).

Sun oil possesses a wax component which requires winterization to remove it. Complete elimination of this undesirable wax through plant breeding is unlikely due to its association with the seed hull, but reduced levels may be achieved. Variations in seed oil wax have been found to depend on the particular hybrid species of sunflower, location of the plant and date of planting. It would appear that the factors influencing the wax content of sunflower are complex (50).

Further plant breeding programs will be concerned not only with the improvement of the quantity and quality of sun oil, but also with the development of species showing lower susceptibility to temperature modification and greater disease- and pest-resistance.

Industrial oils. Non-edible industrial oils make up a significant proportion of the total fats and oils industry (6). Both castor bean and flaxseed have relatively high oil yields, approximately 45% and 35%, respectively (51), yet increas-

ing use of edible oils is being made for industrial purposes. This usage in part reflects the greater availability and better economics of oils such as soybean, sun and palm. However, the possible use of edible plant oils as diesel fuels could put pressure on the availability of these oils (6). Recently, more attention has been paid to new plant sources of fats and oils (7) for the oleochemical industry, rather than improving the oil production of castor bean and flax through the application of biotechnological procedures.

Microbial fats and oils. Microorganisms can play a central role in biotechnology (1). The general ease of culture, reproducibility and potential genetic manipulation of microbes make them ideally suited for the commercial production of a variety of materials including fats and oils. Conditions for the handling and growth of microorganisms, as required for specific product formation, have been described (52). Product formation is favored by the fact that microbial cell generation times are short and can permit substantial physiological manipulation. In addition, the introduction of new desirable strains of microorganisms through genetic engineering has tremendous potential, although several technical difficulties do exist (53). Application of microbial systems to the formation of many products would appear to be most promising. While microorganisms have long been regarded as possible sources of fats and oils (54), only limited application has been made on an industrial scale so far. Certain microorganisms possess a high capacity to form and accumulate triacylglycerol. These high oil-accumulating species have been referred to as oleaginous microorganisms and the product of their activity as single cell oil (SCO) (54, 55). Arbitrarily, the term oleaginous indicates the potential occurrence of fats, oils and allied compounds at a level >25% of the biomass (54). Values ranging up to 70% have been noted. In general, oleaginicity is to be found more frequantly with yeasts (54-56), fungi (54,57) and algae (54,58) than with bacteria. Exhaustive examination of all microorganisms for an oleaginous nature has not been made. New species and mutant strains await discovery and recognition. Conventionally, the identification of an oleaginous microorganism can be made by microscopic examination of the cell cytoplasm for oil droplets. Enhanced visualization can be made by staining with an appropriate dye such as Sudan Black or Nile Blue A. Alternatively, the characteristic presence of ATP:citrate lyase (EC 4.1.3.8) has been suggested as a means of detecting oleaginous organisms in the absence of any oil accumulation (59). Consideration also must be given to the extreme importance of environmental growth conditions on the quantity and quality of the fats and oils produced (54,56-58). Fat and oil production and accumulation, when carbohydrate is substrate, usually is favored with a high carbon:nitrogen ratio in the growth medium (54).

Bacterial oil. Oleaginous bacteria usually accumulate complex lipids rather than triacylclycerols in the cell envelope, which creates difficulties for their subsequent extraction (54). Thus the potential application of bacteria may be limited. Nonetheless, a high content of oil has been observed in *Arthrobacter* AK 19 grown on glucose (60). The production of specialty waxes by bacteria will be discussed later.

Yeast oil. Conditions have been described for high oil production (40-65% of the biomass) in certain oleaginous species including members of the genera *Lipomyces, Rhodotorula* and *Candida* (54-56). While subject to modification by environmental factors, the general fatty acid distribution in the triacylglycerol component resembles that found in plant oils (54,56,61). In particular, saturated-unsaturated-saturated (SUS) and saturated-unsaturated-unsaturated (SUU) types predominate. Thus the oil produced by *Lipomyces starkeyi* has been noted to resemble palm oil (62,63). Particular production of SUS type oil as a possible cocoa butter substitute has been investigated with *Rhodosporidium toruloides, L. starkeyi, L. lipofer, Rhodotorula glutinis* and *R. graminis* (64). These productions, however, require isolation and processing of the oil to yield the desirable SUS type triacylglycerol. Scope still exists for the selection of yeast and conditions of growth to provide more abundant supplies of this potential cocoa butter substitute. Consideration has been given to the possible modification by yeast fermentation of low-cost tallow or lard as substrate into an acceptable cocoa butter substitute (61,65). Only limited success has been obtained so far with *Candida lipolytica*, but adoption of specific growth conditions and the substitute use of thermotolerant yeast may provide a higher yield of the desired product (65). The economics of the procedure, however, would appear to be potentially very high.

Factors associated with the accumulation of particular fats and oils, and the formation of specific triacylglycerols, still await complete elucidation. The selection of appropriate yeast strains for the incorporation of desirable fatty acids at definite positions on the glycerol moiety of triacylglycerols is largely dependent upon a better comprehension of the biochemistry involved. Fatty acid composition can be modified by alteration of growth factors such as temperature. In certain limited cases, major changes in fatty acid composition may be achieved from the growth of yeast (and bacteria) on *n*-alkanes (56,66). The oils so produced, however, would not find acceptance for food use but could well meet certain industrial demands.

The efficiency of fats and oils production from yeast growth on carbohydrate has been calculated to have a theoretical value of 33%, although, in practice, a conversion of >22% is seldom achieved (54). An optimal rate of oil production of 0.09 g oil/g non-fat yeast/hr also has been obtained, but under conditions which would not be economical. Given favorable engineering considerations for large-scale production, a cheap, readily available carbon source as substrate, and a market for the yeast protein by-product as animal feed, SCO conceivably could compete on an economic basis with plant oils. A particularly advantageous situation could be argued for specialty oils such as cocoa butter substitute.

Fungal oil. Oleaginous fungi do exist but have not been extensively studied (54). Those organisms which have received the most attention include members of the genera *Mucor, Penicillium, Aspergillus* and *Fusarium.* Usually a prolonged period of growth is required for oil accumulation which is often not of the conventional triacylglycerol type. Under these conditions a potential problem may arise with the development of mycelia mats of the fungi. Thus any possible adaption of such a system to scale-up will require specially engineered fermenters to prevent damage to the elaborate fungal structure. The general response of fungi to continuous culture in stirred (aerated) fermenters has still to be determined but may result in enhanced oil production (54).

Specific analysis of the triacylglycerol component of *Phycomyces blakesleeanus* revealed a predominant composition of SUS, SUU and UUU types and thus resembled that of a conventional plant oil (67). The particular effects of environmental factors on fungal growth have not been described in detail. The quantity and quality of the oils produced by *Tolyposporium ehrenbergii* and *Sphacelotheca reiliana* have been found to be markedly influenced by the nature of the carbohydrate substrate and nitrogen source (68). In particular, the fatty acid composition was influenced by a variety of carbohydrates obtained as industrial by-products. The true potential of fungi in oil production has yet to be established.

Algal oil. Several species of algae and diatoms have been

determined to be oleaginous (54,58,69,70). Fat and oil production can reach a value of 70% of the biomass in certain species. Various conditions of growth can influence the accumulation of lipid. In particular, production is favored by nitrogen starvation (58,69,70), elevated temperature (58) and general conditions of stress to the algae (71). Analyses of the lipid composition have been limited, but the occurrence of triacylglycerol in the range of 60-80% of the total lipid has been observed (53,71,72). Algal oils generally are characterized by a relatively high content of polyunsaturated fatty acids which may be of potential significance in human nutrition (54). Unfortunately, this component may tend to impart a "fishy" taste to the oil and thus affect its acceptability for use in foodstuffs. The particular fatty acid composition of the triacylglycerol produced by *Neochloris oleoabundans* (71) reveals a higher saturated acid component (25%) and C_{20} acids (5%) than is generally found with conventional plant seed oils (50). Algal oils also differ from conventional plant oils by possessing a higher content of non-saponifiable matter and phospholipids (54). Processing to remove these compounds would be a probable requirement to permit the use of these oils.

Various advantages and disadvantages have been advanced regarding industrial-scale utilization of algae for the production of consumer products including fats and oils (54,58,72,73). The potentially high lipid production obtained from the use of appropriate species and strains, and growth conditions in laboratory studies have yet to be transferred successfully to large-scale open pond systems. Calculations can be made that oil production by certain microalgae on an area basis could be 30 times greater than that for soybean production (58). In open systems under natural conditions, however, a much reduced algal production of perhaps 10% would be anticipated (54), although this could still compete with seed oil production, all other factors being equal. Also possibilities do exist for genetic improvement of algal oil production under outdoor open systems (58). Some additional difficulties exist for large-scale use of algae including the ever-present problem of contaminant growth and the economics of harvesting (73). Coupling of lipid production to some alternate application of algae such as the treatment of sewage and waste water might be more economically viable (54,73). Oil so produced, however, would be unacceptable to the food industry but could find a use as an energy source or as an oleochemical (58,70). As yet the full potential for oil production from algal growth on an industrial scale has not been determined.

BIOCATALYZED SYSTEMS

As previously discussed, the production of fats and oils through the metabolic activities of intact whole cell systems of animals, plants and microorganisms forms the basis of the so-called oil technology. The new technology, associated with the utilization of isolated non-growing single cells and enzyme preparations, is gaining favor as a means of product modification and formation of certain specialty (secondary) products including fats, oils and derivatives (see Fig. 3). This procedure exploits the unique ability of biocatalysts to catalyze specific chemical transformations under mild reaction conditions. Cell-free enzyme systems have many advantages over conventional chemical processes where a number of sequential reactions may be required. Microorganisms have proved to be a particularly attractive source of enzymes. In particular, genetic manipulation of microorganisms may permit the production of highly desirable enzymes in high yields. The use of isolated whole cell systems, however, obviates the costly procedure of enzyme isolation and purification, although the procedure is generally less efficient.

Two general procedures are available involving either a

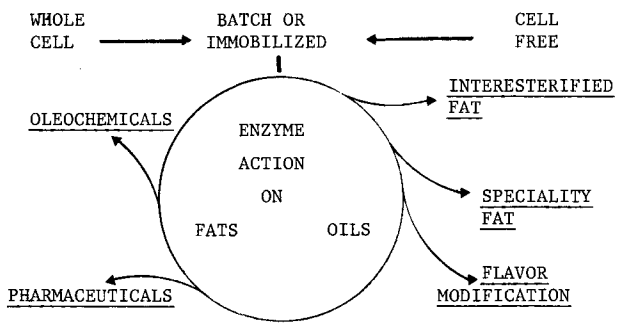

FIG. 3. Enzyme systems and the production of select fatty products.

TABLE II

Major Advantages and Disadvantages of the Application of Immobilized Systems in Biotechnology

Advantages	Disadvantages
1. Continuous operation—flow-through system	
2. Ease of regulation	
3. Re-use of biocatalyst	
4. Improved biocatalyst stability	Reduced biocatalyst efficiency
5. Possibility of multi-biocatalyst systems $(S \xrightarrow{E_1} P_1 \xrightarrow{E_2} P_2 \xrightarrow{E_3} P_3)$	
6. Reduced effluent disposal problems	
7.	Difficulties with enzyme co-factor-dependent systems
8.	Variations in engineering design

batch, free system or an immobilized system. In the former system the cells or enzyme preparation are uniformly dispersed components of the reaction. The more recent introduction of immobilized systems involves the physical restriction of cells or enzymes over which the reaction medium passes. Considerable advantages exist with immobilized systems and are listed in Table II.

Several immobilization procedures are available including physical entrapment or chemical bonding to some inert support. Whole cells or enzyme preparations may be entrapped within a gel or encapsulated within a microcapsule (74-76). Entrapment in natural or synthetic gel matrices is an approach of high potential (76). The procedures can be effectively carried out by polymerizing some prepolymer, e.g. urethane in the presence of cells or enzyme. Of particular relevance is the capacity to modify, among other factors, the hydrophilic-hydrophobic balance of the gel to favor bioconversion of hydrophobic compounds by entrapped biocatalysts (76). Alternatively, systems may be chemically immobilized through covalent attachment to solid supports or by cross-linking. In general, higher enzyme activity is obtained by this procedure, but the preparation process is difficult (77).

Enzyme Applications

Oil extraction. Non-lipolytic enzymes have been used to enhance extractability of oil from seeds (78,79). A variety of microorganisms can serve as potent enzyme sources. Development of a laboratory-scale procedure involving enzyme treatment of ground soybean and extraction with hexane gave a 90% oil recovery compared with conventional crushing and extraction (78). Several advantages exist with the procedure. Lowered temperatures (and hence lower costs) are involved, and the recovered oil meets quality standards that may not require so much further processing (78).

Fatty acid, mono- and diglyceride production. Complete release of fatty acid from triglycerides can be achieved enzymatically (79-81), and has been the subject of many patents (82). The rate of hydrolysis varies with the nature of the oil, but under appropriate conditions complete release of fatty acid can be accomplished within 3 hrs (80,81). Thus, this procedure may prove to be more economical and give greater energy conservation than conventional practices for the splitting of fats and oils (39). Lipases, showing varying specificity for fatty acid location on the glycerol portion of the triacylglycerol and for the fatty acid nature are known (80-84). In particular lipases from *Candida rugosa (C. cylindracea), Aspergillus niger, Rhizopus arrhizus* and *Geotrichum candidum* are commercially available. Appropriate choice of enzyme and incubation conditions can result in the production of different mono- and diglycerides with the release of fatty acid. Attempts to use lipase from *C. cylindracea* entrapped with a hydrophobic photocross-linkable resin prepolymer have resulted in an activity of only about 30% of the free enzyme. Thus, the gain in operational stability of the enzyme was made at the expense of its catalytic efficiency (85).

Glyceride formation. Thermodynamically, the reversal of the hydrolytic reaction catalyzed by lipase will be favored by the removal of water. Many earlier studies did not recognize this factor sufficiently. However, reaction of fatty acid in the presence of excess glycerol and lipase derived from either *A. niger* or *R. delemar* yielded mono- and diglycerides; triglyceride was not a product under these conditions (86). Similar glyceride formation also has been obtained on passing a solution of glycerol and fatty acid in diisopropyl ether-acetone over a column packed with dried defatted mycelia of *R. arrhizus* (87). Formation of all theoretically possible glyceride types on incubation of excess fatty acid and lipase obtained from *A. niger* or *C. rugosa* has been determined when removal of water from the system was made (80). In general, the esterification process proceeds slowly but has the advantage of taking place at ambient temperature and without having to resort to the use of fatty acyl halides. Production of triacylglycerol in greater than 70% yield is a very slow process involving over 6 weeks of reaction (80). Nonetheless, production of glycerides by *Chromobacterium viscosum* lipase in a microporous membrane bioreactor has been achieved with results exceeding 70% over a period of 1 mo (88). The major products of reaction were mono- and diglycerides.

Lipase also has been used to affect modification of the fatty acid composition of triglycerides through acyl exchange under conditions of minimal water concentration (83,84,89,90). In particular, use has been made of lipase from *R. delemar* to catalyze the exchange of oleic acid for stearic acid in triolein. Although bioconversion of lipophilic compounds by immobilized systems is favored by using organic solvents (83,84,90,91), the addition of quartz sand or celite as a disperser was found to be necessary in order to obtain a high degree of acyl exchange (89). Under these conditions, values of up to 30% acyl exchange were obtained. It would appear that the activity of the acyl exchange is closely related to that of glyceride synthesis (89). Exploitation of the varying specificities of different lipases may permit production of a variety of desirable glyceride mixtures which would, otherwise, be unobtainable using conventional chemical interesterification processes (83,84).

Interesterified fats are of commercial interest for production not only of margarine and shortenings, but also of specialty fats. Thus, for example, cocoa butter-like fat has been produced enzymatically from olive oil and stearic acid (89,90). Particular formation of 1-palmitoyl-2-oleoyl-3-stearoyl *rac*-glycerol, the major triglyceride of cocoa butter, has been obtained on reacting oleic anhydride with 1-palmitoyl-3-stearoyl *rac*-glycerol in the presence of lipase from *G. candidum* (92). Further developments in this area can be fully anticipated.

Steroid transformations. The pharmaceutical industry has been and is making extensive use of biotechnology for production of specific steroids of high commercial value, including estrogens and various corticosteroids. Selective transformations in high yields have been the basis of the numerous applications (82,93,94). Both microbial (95,96) and plant sources (97,98) have been used as sources of the specific biocatalysts for particular reactions. Special emphasis presently is being placed on the application of immobilized systems (94-96,98,99). Consideration also has been given to present and future synthetic routes for steroid production via fermentation procedures (82,93,94).

The general procedures for production of a particular product has been to use a biocatalyst system to form a specific steroid intermediate which is, in turn, subjected to further chemical modification (93). Major attention has been paid to biocatalysts for specific reactions involving sterol side-chain cleavage, reductions, hydroxylations, isomerizations, etc. (94-97). Thus, for example, cholesterol can be converted to 20-carboxy-pregna-1,3-dien-3-one, in 95% yield, by a mutant of *Corynebacterium* sp. (100), and prednisone recovered following further microbial and chemical treatments (82). Oxidation of androsterone to androstanedione can be accomplished using immobilized purified enzyme and immobilized NAD^+ (101). Such systems tend to be more complicated and expensive to operate in a continuous manner due to the need for special immobilization practices and regeneration of the enzyme co-factor. The small-sized enzyme co-factors may be conveniently immo-

bilized by using lipid-polyamide membrane microcapsules (102). Re-oxidation of NADH can be obtained by incorporating a methoxy derivative of phenazine methosulfate into the system (101). Alternatively, utilization of immobilized *Aspergillus phoenicis* has allowed for the simultaneous specific hydroxylation of progesterone to 11-hydroxyprogesterone and cofactor regeneration (101). Continuous operation of such systems over a period of 25 days has been obtained. Several factors are known to limit transformations in column type reactors including cell type, substrate concentration, oxygen requirement, and nature of the immobilization support (95,96). Particular attention must be paid to the use of organic solvent systems and the immobilizing gel hydrophobicity to allow for efficient steroid bioconversion (101,103,104).

Fatty acid modification. Particular fatty acids of commercial interest can be obtained either directly from selected biological sources or by biocatalytic conversion of common fatty acids. The techniques of biotechnology are available for fatty acid transformations but have not been generally applied. Thus desirable unsaturated acids generally are obtained from an appropriate natural source rather than converting saturated fatty acids by specific desaturase systems. The reverse process of biohydrogenation, which could be of industrial interest in the processing of vegetable oils to margarine, shortening, etc., would be difficult to implement. While the rumen bacterium *Butyrivibrio fibrisolvens* has been found capable of biohydrogenating linoleic acid, it is necessary to conduct the reaction in the absence of oxygen and the presence of α-tocopherolquinol as reducing source (105). Furthermore, the ultimate product of reaction is *trans*-11-octadecenoate, which may be considered to be an undesirable component in the diet of man (106).

Enzyme-catalyzed attack of unsaturated fatty acid, while creating potential problems in e.g., the flavor reversion of soybean oil, has been applied to the deliberate production of fatty acid hydroperoxides. In particular, immobilized soybean lipoxygenase has been employed in the continuous production of 13-hydroperoxylinoleic acid and 15-hydroperoxyarachidonic acid (107). The possibility of adapting different lipoxygenase reactors to obtain double dioxygenated arachidonic acid is under investigation. These compounds are of potential industrial significance for the synthesis of prostaglandins and allied compounds (108,109). The derivation of flavor components in foodstuffs through microbial oxidative (and lipolytic) action on lipids is well recognized (79,110). Commercial application of immobilized organisms for these purposes has, so far, been limited (111).

Other modifications of fatty acids to yield hydroxy or dioic acids may be contemplated. An industrial market exists for such products (7,112). The possibility of microorganisms converting *n*-alkanes to these products has been discussed (113,114). Future application of the general technology employed in biocatalyzed steroid transformations can be envisioned. Thus, immobilized cells of *C. tropicalis* have been employed for the production of ω-hydroxy acids and α,ω-dioic acids (115). A system present in *C. rugosa* is also capable of producing D-β-hydroxy acid from short-chain carboxylic acid (116).

NEW SOURCES OF INDUSTRIALLY DESIRABLE FATTY ACIDS

Certain fatty acids, possessing unique chemical and physical properties, can have a high industrial potential. Production of these acids from natural sources has many advantages over direct chemical synthesis (7,39). However, before application of the new biotechnology can even be considered, information must first be obtained on the specific occurrence, if any, of these compounds. Screening of organisms for such information is essential but time-consuming and tedious. Thus, a 25-yr long program has been conducted by the USDA in which over 6,500 species of wild plants have been examined as sources of desirable oils (7,117,118). A presumably greater potential exists with microorganisms, particularly yeasts, fungi and algae. Detailed studies have so far been limited but nevertheless should be undertaken. The topic of new sources of fats and oils, including less common fatty acid components, has been discussed extensively (7,117,118). A general comparison of some plant and microbial sources for various fatty acids of potential indus-

TABLE III

Some Sources of Industrially Desirable Fatty Acids (Literature citations are given in parentheses)

Acid		Source	
Class	Example	Plant	Microorganism
Short-chain	C_8-C_{10}	*Cuphea (Lythraceae)*(119,120)	Fungus *(Entomophthora coronata)*(54,121)
Medium-chain	Lauric acid	African oil palm *Elaeis guineensis)*(46)	Fungus *(Entomophthora coronata)*(54,121)
		Coconut palm *(Cocos nucifera)*(46)	Fungus *(Entomophthora obscura)*(122)
Long-chain	Erucic acid	*Crambe (Crambe abyssinica)*(123)	Yeast *(Rhodotorula rubra)*(124)
		Rapeseed *(Brassica napus)*(42)	
Polyunsaturated	α-linolenic acid	Flax *(Linum usitatissimum)*(50)	
	γ-linolenic acid	Evening primrose (*Oenothera* spp.) (125)	Alga *(Spirulina platensis)*(54,127)
		Nonnea macrosperma (126)	Fungi *(Phycomycetes)*(54)
	20:5ω3	–	Alga *(Chlorella minutissima)*(128)
Hydroxy	Ricinoleic acid	Castor bean *(Ricinis communis)* (50)	Fungus *(Claviceps purpurea)*(54,130)
		Linum (*Linum* spp.)(129)	
Epoxy	Vernolic acid	Stokes aster *(Stokesia laevis)* (131)	Yeast (*Lipomyces* sp.)(134)
		Vernonia galamensis (132)	
		Vernonia volkameriaefolia (133)	

trial significance is given in Table III. Further examination of these and other sources coupled with the considered application of biotechnology should supply valuable quantities of the desired products.

SPECIALTY PRODUCTS

The conventional growth of crops, subject to improved germplasm and agronomic practices, will continue to provide the bulk of desirable oils. It probably will be in the area of specialty products that biotechnology will have its major impact. Examples of specialty products are several and cover a wide range of industries, but only a few will be discussed here. In general, these products have a highly specialized market and command a market monetary value which may justify the economics of biotechnology. Usually such applications should show a price differential of at least $500 per kg between starting materials and product (21).

Cocoa Butter Substitutes

The fat (butter) obtained from beans of the cacao tree *(Theobroma cacao)* is used principally in chocolate and confectionary manufacturing, although some specialized applications in the soaps and pharmaceuticals industries also exist. Its price is the highest among all commercial fats and oils and may fluctuate widely (54,135). The particular desirable properties of cocoa butter include its characteristic melting behavior (136), but for economic reasons and uncertainty of supplies cocoa butter substitutes have been sought (135).

The major component of cocoa butter is triacylglycerol of the types SOS and particularly 1-palmitoyl, 2-oleoyl, 3-stearoylglycerol and 1,3-distearoyl, 2-oleoylglycerol (136). Cocoa butter substitutes of similar but not identical compositions have been obtained from certain plant sources. These fats have included illipe butter from kernels of *Bassia longifolia* (135) and mango fat from seeds of *Mangifera indica* (137,138). Consideration of more reliable sources of hard fats has been given to derived or chemically-modified fractions from more conventional oils such as palm kernel, coconut and soya. The processing steps involved, e.g., solvent fractionation, hydrogenation and interesterification, introduce a significant cost factor. Alternative applications of plant systems are under investigation. In particular, the possible use of in vitro cultures of callus tissue or cell suspensions (139-141) and asexual embryos (142,143) of cacao has been examined. The temperature of cultivation, among other factors, has been found to markedly influence the fatty acid composition of the triacylglycerol component (141,144). Thus, strict regulation of the environment temperature will be of prime importance in any commercial use of these systems. It is believed that asexual embryos grown at 26 C may, if allowed to continue to maturity, produce a fat closely resembling that of mature fieldgrown zygotic embryos (144). Further studies on this approach to producing the desired fat would appear to be warranted, more particularly since the product could be legitimately labelled cocoa butter.

As previously discussed, cocoa butter substitutes may be obtained from the growth of certain yeasts under appropriate conditions or by application of particular enzyme systems. In either case, processing steps will be required and will involve an unknown cost factor. Consequently, the economic feasibility of production by these methods is not available. However, it may be predicted that any substitutes not produced from cacao cannot presumably be designated cocoa butter and consequently will command only a fraction of the price of the authentic material (54).

Plant Lecithin

Phospholipids, and particularly commercial lecithin, have wide applications in food systems, industrial uses and certain medical treatments (145-147). The principal source of commercial lecithin presently is the soybean, although the other seed crops including cotton, corn, rape and sunflower also are being considered. The development of glandless (or gossypol-free) cottonseed cultivars should now allow for easier recovery of lecithin and hence increase its marketability from this source (147).

Lecithin obtained by degumming of soybean oil is a mixture of various phospholipids, oil and sterols (145,148). The relative concentration of the several phospholipid classes has pronounced effects on particular biosurfactant activities. At present, modification of lecithin to obtain the desired properties, e.g. definite oil-water emulsifying behavior, can be achieved by chemical, physical or enzymic means (146). A more satisfactory alternative approach could be to induce the plant into producing directly products possessing the wanted activities. So far, specific attempts to augment or modify the lecithin component by plant breeding or agronomic practices have been limited. Certain plants, e.g. cottonseed or corn, which produce lecithin essentially devoid of linolenic acid, may prove to be a preferred source. Such a product would show good oxidative stability during processing and subsequent utilization (147).

Microbial Biosurfactants

Several lipids produced by microorganisms exhibit surface active properties widely differing from synthetic surfactants (149). Consequently, these products have a potential for many industrial uses including flocculation, emulsification and tertiary oil recovery processes. A particularly attractive feature of these compounds is that they are biodegradable, which minimizes the problem of environmental pollution.

The general chemical nature and physiochemical properties of microbial biosurfactants have been considered in detail (149,150). These surface-active agents frequently are extracellular products of select microorganisms or integral components of the microbial cell surface. Thus, the ability of bacterial suspensions to act as de-emulsifiers of a wide range of synthetic and commercially important emulsions reflects the hydrophilic/lipophilic nature of the cell surface (151). The excreted biosurfactants often are glycolipid in nature, and include trehalose lipids, rhamnolipids, sophorose lipids, diacyldiglycerides and various ill-defined polysaccharide-lipid complexes (150). These lipids are generally produced by certain members of the genera *Arthrobacter, Nocardia, Corynebacteria* and *Torulopsis*. Biosurfactant excretion requires the growth of the microorganism on some hydrocarbon (*n*-alkane) or triacylglycerol substrate (149,152-154). Thus, growth of different strains of *Torulopsis bombicola* on glucose and vegetable oil has given biosurfactant yields of up to 130g/l (153,154). It has been calculated that with a yield of 70g/l there would be 35% conversion of substrate. Furthermore, the cost of production of this mixture of glycolipids would be about $2.10/kg which would be most competitive with that for synthetic surfactants, e.g. span 60 (154). Several multiorganism strategies have been examined for improving biosurfactant yields from wastes (155). In particular, consideration has been given to using CO_2, generated from anaerobic fermentation of municipal wastewater, as substrate for the growth of oleaginous algae. The SCO so produced could then be used to stimulate increased glycolipid biosurfactant production by *T. bombicola*. Such a process would allow not only the bulk production of useful biosurfactant at low cost, but would also improve the economics of waste treatment (155).

Ionic biosurfactants as represented by phospholipids are not generally excretory products of microorganisms although certain exceptions occur with members of *Corynebacteria, Thiobacilli,* and *Candidae* (150). These systems have not been exploited commercially. Ability of the microorganism to excrete the desired product is of prime importance since it allows for easy harvesting and hence lower costs. Further applications of mutants of *Candida lipolytica* which are known to excrete long-chain fatty acids (an alternate biosurfactant) can be contemplated (156).

Waxes

A need for additional sources of waxes (long chain fatty acid esters of long chain alcohols) exists (7), and may be met by the appropriate application of biotechnology. Since the general ban on the import of sperm whale oil into the U.S. was introduced in 1971, new sources of commercially desirable waxes have been sought. Although the oil from certain deep water fish species, including orange roughy *(Hoplostethus atlanticus)*, has been considered as a possible substitute for sperm whale oil (157), production will be limited by legislation restricting the extent of fishing. Introduction of some form of aquaculturing of orange roughy presumably would be acceptable. More attention, however, has centered on the seed oil component of the desert shrub, jojoba *(Simmondsia chinensis)*, for which several industrial uses have been cited (158,159). Although the wax composition is primarily *cis*-13-docosenyl *cis*-11-eicosenoate, the general properties of the total mixture are similar to those of spermaceti (159). Attempts to increase oil productivity through improved agronomic practices and plant breeding programs are in progress but are handicapped by the prolonged period of 10-12 years before new plants reach full maturity (158). Synthesis of other desirable waxes from triglycerides derived from meadow foam *(Limnanthes alba)* (7,160) and crambe *(Crambe abyssinica)* (7,123) also has been considered. Further biotechnological development of these and other plant sources is necessary to ensure favorable wax production. Cell suspensions of soy and rape have been found capable of converting long-chain alcohols to waxes (161).

Microbial production of waxes is known and is generally more versatile. Switching of the growth of *Euglena gracilis* from aerobic to anaerobic conditions can result in significant conversion of storage polysaccharide to wax. This material can then amount to 49.9% of the cell weight with a yield of 2.1g/l (162,163). Relatively short chain saturated waxes, primarily of C chain length of 28, 27, 29 and 30, have been determined (162,163). Modification of the wax composition has been achieved by varying the nature of the C-substrate in the growth medium (163). Longer chain waxes of C chain length predominantly 36,34 and 32 can be produced by the bacterium, *Micrococcus cryophilus* (164). Manipulation of the growth temperature has been found to alter the wax composition. Thus, lowering of the growth temperature increased the degree of unsaturation but decreased the C chain length of the wax. Detailed studies have been made on *Acinetobacter* sp. (also known as *Micrococcus cerificans)* for the production of waxes as sperm oil substitutes (165-168). The procedure has been patented (169). In general, C_{32} - C_{42} waxes can be produced varying in composition dependent upon the chain length of the C-substrate and the growth temperature. Capillary gas chromatography has revealed the presence of both saturated and unsaturated waxes (165,166,168). Growth of the bacterium on individual C_n*n*-alkanes results in the primary production of C_{2n}, C_{2n-2}, and C_{2n-4} waxes (165-167). A variety of waxes can arise from growth on a mixture of *n*-alkanes, e.g. primary gas oil (168) or short-chain (C_2 or C_3) acids and alcohols (167). Growth at 30 C compared wi[redacted] crease in the degree of unsaturation [redacted] duction has been found to be enhanced [redacted] immiscible *n*-alkane substrate is used (16[redacted]

Waxes also can be produced enzymatic[redacted] esterification of alcohols under condition[redacted] favor the hydrolytic process. Resting and[redacted] cells of *Corynebacterium* sp. have been found [redacted] of forming a variety of esters but to differing de[redacted] Thus waxes, e.g. oleyl oleate, oleyl palmitate for[redacted] whereas methyl and ethyl fatty acid esters did no[redacted] tions involving *n*-hexane as solvent favored wax fo[redacted] Similarly, organic solvent enhanced cetyl linoleate [redacted] tion in a continuous reactor system involving a c[redacted] packed with dried defatted mycelia of *R. arrhizus* (87[redacted]

Prostaglandins and Allied Compounds

The increasing importance of prostaglandins and all[redacted] derivatives as pharmaceutical agents is requiring improve[redacted] specific methods for their commercial production. Variou[redacted] microbial systems have been examined (171). The technology for these practices should be generally similar to the procedures being developed for steroid transformations. A procedure of possible importance has been described using the prostaglandin synthetase complex of ram seminal vesicles immobilized in photocrosslinkable gel to convert arachidonic acid to PGG and PGE (108,109). An economical source of arachidonic acid would appear to be production by the red alga *Porphyridium cruentum* (172).

DISCUSSION

From the preceding discussion, it is obvious that the techniques of biotechnology, both old and new, are finding applications in the fats and oils industry. Further potential for biotechnology in this field exists, but it will be restricted by practical considerations, economics and legislation.

Conventional crops and agricultural practices will continue to provide the bulk of the edible oils market in the foreseeable future, but may ultimately be unable to meet the projected increase in demands (3,6). More intensive research is required to allow for increased oil productivity by plants (173). Areas of possible activity have been presented in Table I. At the same time development of alternate sources must be considered. Theoretically, all other factors being equal, algal ponds could be much more efficient than the present oil-producing crops (58).

Several factors have to be considered when assessing the relative merits of plants and microorganisms (Table IV). Environmental effects can markedly influence the growth and genotype expression of both plant crops (15) and isolated cells (174). Plants show varying responses to the environment which may affect both quality and quantity of oil produced. Control of the weather is neither practical nor economical. It may be possible through genetic engineering to produce more resistant cultivars (11), but there is a potential danger inherent in the development of a limited genetic base. The problem of regulation of pest infestation is also of considerable concern and importance.

Continuous culture of isolated cells in the chemostat-type system allows the definite regulation and manipulation of various environmental factors. Such a practice is amenable to microprocessor control as has been applied to the production of oil by *Lipomyces starkeyi* (175). Experience gained in the fermentation industry should be valuable in combating the ever-present problem of microbial contamination of the system.

Plants show very slow growth compared with microorganisms. This factor has a bearing on both crop production

[redacted]rison of the General Factors Influencing the Application of the Techniques [redacted]chnology to Plants and Microorganisms

Factor	Plant		Microorganism
	Intact	Isolated cell	
Cultivation including regulation of the environment	Variable	Certain problems	Easy
Growth needs	Several	Several	Low
Generation time	Prolonged	Slow	Rapid
Genetic engineering	Several possibilities	Unknown potential	Unknown potential
Productivity	Generally low	Low	Potentially high
Product application	Several possibilities	Specialty product	Specialty product
Engineering	Low	High	High
Economics	Low	High	High

and development of new lines. Thus, for example, the jojoba plant takes 7 yrs to produce oil-bearing seeds and 10 yrs to reach maturity (158). Development, testing and commercial release of triazine-resistant canola was rapid but still took 6 yrs (W. D. Beversdorf - personal communication). Isolated plant cells also show a much longer doubling time when compared with yeast. In addition to shorter development times for new desirable cell lines, the more rapidly generating microbial cells have the possibility of greater oil productivity (54,55,58).

The most efficient applications of genetic engineering will be, to a large extent, dependent upon the availability of more detailed knowledge of the biochemistry, physiology and genetics of the cell. In particular, more definite information on the genetic control of the biochemical processes associated with fat and oil synthesis is required. The complexities of multiple gene systems within a metabolic pathway as well as multiple gene/multiple gene product systems are generally recognized (176). Additional manipulation of oil quantity and quality may be contemplated through genetic engineering to complement the advances being made through plant selection and breeding programs (8). Applications to microbial systems are less clear. Development of oleaginous yeast strains that could use cheap carbohydrate substrates or methanol merits attention (54).

Various desirable oleoproducts can be derived not only from plants but also from microorganisms (Table V). Although microorganisms might appear to have greater potential to the industry than is now appreciated, it is in the area of specialty products where the greatest efforts probably will be made. Increased production of these compounds may be anticipated from further application of immobilized cells and enzyme systems. The actual industrial implementation of these biotechnological procedures will be governed by engineering and economic considerations.

Space limitation does not permit a discussion of the importance of various engineering aspects associated with possible industrial adoptions of biotechnology. Particular attention will have to be focussed on the further development of biological reactors (177) and on the definite problems involved with scale-up of plant (22,23) and microbial cell systems (178). A considerable cost factor can be anticipated. Thus the specialty product will have to command a high market value to justify the investment in engineering design and facilities. On the other hand, bulk quantities of fats and oils probably will continue to be derived from field crops which involves a minimal engineering component.

TABLE V

A Comparison of Oleoproduct, Potential Use and Oleoproductivity of Plants and Microorganisms

Oleoproduct	Potential use	Potential oleoproductivity	
		Plant	Microorganism
Oil	Diesel fuel	+	–
	Food	+++	–
Specialty oil	Food	++	++*
Hydrocarbons	Rubber	+++	unknown
Fatty acids	Feedstock	+++	+
Waxes	Feedstock	+++	++
Glycolipids	Surfactants	–	++
Lecithin	Surfactant	+++	+
Steroids	Pharmaceuticals	++	++*

+++ = very high; – = low.

*Immobilized microorganism or enzyme system.

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