Biotech conference draws 500 persons

Approximately 500 technical registrants and company exhibit representatives attended the World Conference on Biotechnology for the Fats and Oils Industry in Hamburg, West Germany, Sept. 27-Oct. 2, 1987.

Primary organizers were the American Oil Chemists' Society (AOCS) and the German Society for Fat Research (DGF), with the DGF holding its annual meeting in conjunction with the international conference. Other participating organizations included the Institut des Corps Gras, the Japan Oil Chemists' Society and the Stazione Sperimentale degli Oli e dei Grassi. The program was prepared under the guidance of Thomas H. Applewhite and Karl F. Gander, who cochaired the conference.

In five days of technical sessions, registrants from 40 countries heard talks ranging from modification of plants and fats and oils through manipulative engineering to regulatory action related to biotechnology. Invited speakers came from the U.S., West Germany, Japan, England, Canada, Australia, Denmark, France and The Netherlands.

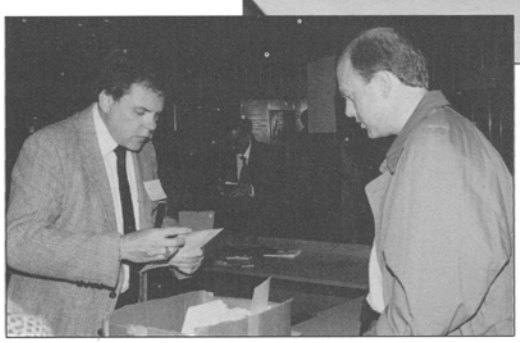
In addition to more than 50 plenary talks, the conference also featured 41 posters presented during three days. "The quality of the posters was excellent," Richard Wilson, poster session organizer, said, noting, "The poster sessions give technical participants the chance to stretch and use their minds at the same time. They can ask questions and get answers." The three separate poster sessions focused on different topics, from biochemistry and plant system genetics on Tuesday, and lipases on Wednesday, to process lipases and industrial methods on Thursday.

The exhibition accompanying the conference featured 26 booths representing 20 companies (see accompanying article).

In opening ceremonies, Dr. von Dohnanyi, president of the Senate and first mayor of Hamburg, and Georg Jung of the Federal Ministry of Food, Agriculture and Forestry welcomed participants to Hamburg and West Germany. After Applewhite formally opened the conference, Gander told participants, "I do hope that this congress will act as a catalyst—in fact, as an enzyme—in enhancing the worldwide progress of modern biotechnology."

Keynote addresses presented in Monday's opening session were given by Paul K. Stumpf of the University of California, Colin Ratledge of the University of Hull and Tsuneo Yamane of Nagoya University. These talks set the tone of the meeting by emphasizing the basic biochemistry and biotechnology required for successful genetic modification to produce fats and oils and their derivatives. The three talks are published as technical news features in this issue and also will be included in the published

Attendees to the World Conference on Biotechnology listen to speakers during five days of technical sessions. Approximately 500 persons took part in the conference.



AOCS Technical Director Dave Berner (left) assists a registrant at the World Conference on Biotechnology for the Fats and Oils Industry, held in Hamburg.

The skyline of Hamburg, as seen from the Hamburg Plaza Hotel.

Normann Medal recipients are Joyce Beare-Rogers (above) and Saburo Fukui (left). Beare-Rogers spoke on "Nutritional Attributes of Fatty Acids," while Fukui presented a talk on "Conversions of Lipophilic Substances by Encapsulated Biocatalysts."

Karl Gander of DGF talks with Normann Medal recipient Joyce Beare-Rogers at the German party held Wednesday evening at the Atlantic Hotel.

proceedings of the conference, scheduled to be available during the first quarter of 1988.

Also in conjunction with the meetings, DGF held its annual Normann Medal ceremonies. This year, DGF honored two scientists, Joyce Beare-Rogers of the Department of Health and Welfare in Ottawa, Canada, and Saburo Fukui, professor emeritus of the University of Kyoto, Japan. Beare-Rogers presented a talk on "Nutritional Attributes of Fatty Acids" and Fukui spoke on "Conversions of Lipophilic Substances by Encapsulated Biocatalysts."

Gander, in making the Normann Medal presentations, called Fukui "a grand old man of biotechnology. Not only does he find the time to do things but also to contemplate the how of doing things." Gander introduced Beare-Rogers as "a very charming lady and well-respected lipid researcher."

Social activities included a visit to Hamburg's Town Hall Monday evening and a Tuesday evening concert of organ music at the St. Petri Church, sponsored by Deutsche Unilever GmbH. The organist was Ernst-Ulrich von Kameke, who performed works by Bach, Brahms, Gigout, Lefébure-Wély and Widor. After the concert, those buying tickets went to the Fleetenkieker restaurant for a German meal. Other social activities included a dinner dance featuring an evening of folk music Wednesday evening at the Atlantic Hotel and a Thursday evening boat trip on the Elbe River. There also was a spouses' program for those accompanying technical registrants. Activities included a tour of Hamburg and tips on where to shop, a boat trip on the Alster Lake, a visit to the open-air museum in Kiekeberg and a trip to the city of Lüneberg.

During the closing day, regulatory aspects for biotechnology were discussed. In his talk, R. Keith Downey of Agriculture Canada expressed concern over governments granting patents for specific characteristics of plants, such as a U.S. patent for high-oleic sunflower. "If you patent the whole characteristic, this could block developing the characteristic by another method or gene," he said.

In concluding remarks, Gander called the conference "a great scientific event" and Applewhite noted that the conference "opened up new vistas for us to explore, and hopefully, to profit from." Recommending that participants, as well as their management, customers and appropriate regulatory sectors, keep informed on what is happening in the field, Applewhite said biotechnology for fats and oils can offer "a great opportunity to serve mankind." However, he cautioned that it must be applied carefully. "If we do not, we will find ourselves bogged down in a morass."

Technical highlights

The following report summarizing highlights from the technical sessions at the World Conference on Biotechnology for the Fats and Oils Industry was prepared by E.G. Perkins of the University of Illinois' Department of Food Science.

Modification of oil plants

R. Theimer of Bergische Universität presented material showing triglycerides in seeds as distinct spherical granules which appear to be surrounded by a single layer of phospholipid. In the developing seed, lipid bodies develop between the lipophilic phospholipid moieties in the endoplasmic reticulum membranes, pushing them apart and budding off, still surrounded by the phospholipid layer, Theimer said.

A review of present knowledge regarding fatty acid synthesis and triglyceride production in oilseeds was presented by A. Stobart of the University of Bristol. The mechanisms whereby oleate moieties enter phosphatidylcholine for desaturation and the polyunsaturated products made available for triglyceride formation also were reviewed.

J.L. Harwood of University College, speaking on fatty acid

synthesis in plant cells, noted that plants catalyze fatty acid formation by different enzymes. Acetyl-CoA carboxylase and fatty acid synthetase are responsible for de novo formation. Acetyl-CoA carboxylase appears to be a high molecular weight multifunctional protein, and the fatty acid synthetase is a dissociable Type II complex.

It was apparent throughout the conference that success in the area of forming new oils with controlled chain length and unsaturation have been a result of traditional selection and breeding methods rather than genetic engineering techniques. However, there was considerable optimism that although more difficult, these techniques ultimately would become more useful and were capable of increased specificity.

In a similar fashion, Paul Knowles of the University of California reviewed the recent advances in plant breeding for oilseed crops and also stressed the importance of breeding methods combined with newer technologies. He warned that plant breeding augmented with modern biotechnology must be merged closely and that it increasingly is being carried out in the private sector which, although resulting in more rapid development, also results in decreased flow of information to the public sector. As indicated by D.J. Guerra of BioTechnica Canada, the molecular genetics of plant lipid metabolism essentially are uncharacterized although the biochemical knowledge is available. The evidence suggests that acyl carrier protein (ACP) plays a central role in regulation of acyl chain length and distribution. He indicated that to potentially engineer triglyceride synthesis, the relationships between ACP-mediated de novo fatty acid synthesis and the Kennedy pathway must be elucidated. It was stressed that ACP gene manipulation in oilseed crops has become a major research focus.

In another talk, L.H. Jones of Unilever Research discussed the problems associated with the commercial development of oil palm clones. Noting that such clones must be field-tested for several years in different environments before commercial selection is made, Jones said several clones in large-scale production have developed abnormalities. Work currently is aimed at production of normal palms.

Y. Sugimura of Kao Corp. discussed the use of biotechnology for coconut improvement. Since coconut palm is propagated from seeds, the genetic variance between seedlings is large. In order to develop clone materials from proven coconut stock, tissue culture techniques were studied. The development of optimum conditions for culturing isolated embryos was reported. Allan Green of CSIRO, Australia,

Karl F. Gander and Thomas H. Applewhite, who cochaired the conference, share an anecdote after sessions.

Dr. von Dohnanyi, president of the Senate and first mayor of Hamburg, welcomes conference participants to Hamburg.

Georg Jung of the West German Federal Ministry of Food, Agriculture and Forestry speaks during opening ceremonies.

Speakers in a technical session on engineering and scale-up in fat and fatty acid biotransformations field questions during a discussion period.

Jim Lambie (left) and Frank Gunstone enjoy the sites along the Elbe River on the Thursday evening boat trip.

presented material on the genetic modification of flax (linseed) to convert linseed oil from an industrial oil to an edible oil by lowering the linolenic acid content. Linolenic acid was reduced to about 1% by treatment of a high linolenic cultivar *Glenelg* with EMS. Two mutants were isolated and recombined into a single genotype. A concomitant increase in linoleic acid to about 62% also was found.

Significant research was presented by U.S. Department of Agriculture (USDA) researcher Niels Nielsen of Purdue University who showed that protein engineering could produce soy protein with increased protein quality. Furthermore, a soybean lacking in lipoxigenase produced a higher-quality oil and meal from the standpoint of flavor. T. Andreasen of Sungene Technologies Corp. reported important information concerning the improvement of *Brassica* and *Helianthus* seed oils. New phenotypes created by discrete mutations are available readily through mutagenesis and semiclonal variation. Seed oils with very high levels of linoleic and oleic acid have been produced.

In discussion sessions, Nielsen indicated that although the function of lipoxigenase in soybeans is unknown, the L-2 isozyle largely is responsible for off-flavor generation in soymilk and meal produced from beans absent in this isozyme. Asked about the extractability and bioavailability of carbohydrates to monogastric animals, he indicated that the pathways of such carbohydrates as stachyose are being studied but no work had been done to improve carbohydrate concentrations in crops. He explained that soybean protein could be used as a model system to evaluate products made by protein engineering and mentioned the improvement of nutritional value by increasing methionine content as an example.

Andreasen indicated that up to 200 plants had to be screened to find lines with differing fatty acid content, and that seed mutagens caused greater mutations when used.

New and unique oil sources and byproducts

About 500 different fatty acids and lipids have been found in the 10,000 species of plants thus far examined, according to a review presented by R. Kleiman of USDA's Northern Regional Research Center. Kleiman indicated there may be a relationship between such compounds and a particular genus, family or order. Such lipids may be useful as chemotaxonomic markers.

G. Röbbelen of Georg-August University reported on the developments of potential new industrial oil crops such as cuphea (to produce medium-chain triglycerides), calendula (to produce trienoic fatty acids with conjugated double bonds), lesquerella (to produce hydroxy fatty acids) and crambe (to produce long-chain fatty acids).

J. Ohlrogge of Michigan State University reported that in developing soybean seeds, both the acyl carrier protein (ACP) levels and amount of messenger RNA for ACP increase during the period of rapid fatty acid synthesis (FAS), suggesting that oil production is controlled at least partially by turning on FAS genes. He reported the construction of a synthetic ACP gene that is being used to examine the structure, function and regulation of ACP at both protein and nucleic acid levels. Further research into the fatty acid synthetase multi-enzyme complexes was reported by E. Schweizer of Friedrich-Alexander University. The complete nucleotide sequences of the multifunctional FAS1 and FAS2 genes from *S. cerevisiae*, the FAS1 gene from *Y. lipolytica*, and the FAS2 gene from *P. patulum* were presented.

Arguments for the technical and economic aspects and feasibility of single cell oil production using yeast technology were presented by R.S. Moreton of Cadbury Schweppes. The economic extraction of lipid from the cell is crucial to process economics, and this appears to have been solved by bead milling dried yeast cells in hexane. Since the production of one ton of lipid requires about five tons of carbon substrate, the production of vegetable oils by yeast fermentation is not feasible economically unless a very cheap source of substrate is available. Higher-priced specialty fats such as cocoa butter, however, may be feasible.

A paper written by Osamu Suzuki of the National Chemical Laboratory for Industry and presented by Hideo Kikutugi discussed an efficient industrial-scale microbiological method for the production of lipid rich

in γ-linolenic acid (GLA) from *Mortierella* fungi. The fungal mass contained from 37–58% lipid with up to 11% GLA.

The potential of a biotechnological source of eicosapentaenoic acid (EPA) from cultured microalgae was presented by D.J. Kyle of Martek Corp. In view of the current interest in the use of EPA in cardiovascular care and other medical areas, the availability of such a fatty acid from sources other than fish oil may become attractive to the industry. In discussion sessions, Kyle indicated that there is a temperature-dependent effect on growth rate and degree of unsaturation produced.

To a query asking why there still is interest in developing cuphea as a source of lauric and myristic acids instead of relying on conventional sources, L.H. Princen, one of the session chairpersons, explained that industry still seeks alternate sources in case of supply problems. Moreton, meanwhile, indicated that cocoa butter from microbial production would be economical only if the price of cocoa butter were higher.

Modification of fats, oils, fatty acids and glycerol

The use of immobilized enzymes has grown rapidly, as indicated by many of the presentations in this session. Many reactions, such as ester interchange and synthesis, need to be carried out in an organic solvent to prevent hydrolysis. Immobilization in or on a support prevents enzyme denaturation by the organic solvent.

A. Tanaka of Kyoto University reported on the interesterification of olive oil triglycerides with stearic acid in water-saturated hexane by *Rhizopus delmar* lipase entrapped with a hydrophobic photo-cross-linkable resin prepolymer after absorption on Celite. The entrapped enzyme was more stable than Celite-adsorbed enzyme. Examples of several other reactions also were given. In discussion sessions, Tanaka indicated there is a relationship between water content and lipase activity and that the structure of the lipases is very difficult to change. However, changes can be produced by using either highly hydrophylic or hydrophobic gels. He added that about 80% of enzyme activity could be recovered.

Consultant W. Linfield stressed that the rate of enzymatic fat hydrolysis directly is proportional to both the logarithm of reaction time and enzyme concentration. Results obtained with *C. rugosa* were presented as well as the economic aspects of enzymic fat splitting.

Further work on more general ester synthesis with immobilized enzymes was discussed by Peter Eigtved of Novo Industri. The presence of diglycerides in palm oil contributes to its decreased stability during heating. Asked the reason for using a derivative in the esterification of galactolipids, Eigtved indicated that polyols were not soluble or miscible with triglycerides, and the reaction rates were very slow at the interfaces of immiscible materials. In addition, the use of a derivative allows the formation of monoester when other groups are blocked.

Work presented by J. Kurashige of Ajinomoto Co. showed that the use of Celite-immobilized Lipase P can convert the diglyceride to triglyceride at water levels of less than 100 ppm and increased triglyceride content from 87% to 95%. In the question period, Kurashige indicated that it is not possible to esterify phospholipids as diglycerides as this was probably a two-step reaction.

Hydroxy acids are useful as precursors for organic synthesis of many important chemical compounds, but laboratory synthesis of these types of compounds are laborious and expensive. Although hydroxy fatty acids occur as part of many triglycerides, ω-hydroxy acids are more difficult to find. F. Meussdorfer of Henkel reviewed this material and the biochemical reactions involved. He indicated that alkane-degrading organisms such as *Candida*-type or *Coryne* bacteria are more suited to hydroxy acid production if they are blocked in the alkane/fatty acid metabolism to prevent further degradation of the formed product. Asked about the enzymes involved in ω-oxidation of alkanes, Muessdorfer said it may be possible to eliminate alcohol dehydrogenase activity and still have alkane hydroxylation.

N. Uemura of Nippon Mining Co. said that the production of dicarboxylic acids from alkanes by fermentation with *Candida tropicalis* is under way. The 13-carbon dibasic acid brassylic acid now is being commercially produced at 150 tons per year by the fermentation method. R. Hammond of Unilever Research discussed the problems involved with the enzymic modification of mid-chain fatty acids, including reactant supply for biocatalytic routes. D. Estell of Genencor reported on the engineering of lipases and proteases for improved transesterification. He indicated that changes in the lipase could alter the transesterification properties of the enzyme.

Preparation of unique fats, fatty acids and biosurfactants

This session demonstrated the many ways in which microorganisms can be used to prepare a variety of lipid materials without the use of advanced biogenetic engineering processes.

R.G. Schmid of Braunschweig Technical University presented material similar to that given by Kleiman relating to the structures of fatty acids as chemotaxonomical markers with the hope that industrial applications of such compounds may be found from these relationships.

The next several papers illustrated the vast potential that microorganisms have in the production of specific fatty acids, or "tailor-made" fat. Hideaki Yamada of Kyoto University spoke on the production of arachidonic and eicosapentaenoic (EPA) acids by microorganisms. A soil isolate, *Mortierella elongata* 1S-4, was found to show a high productivity of arachidonic acid. The production of arachidonic acid reached 3.6 g/l (80 mg/g dry cells) when the fungus was grown in a medium containing 2% glucose and 0.5% yeast extract as main carbon and nitrogen sources. The fungus produced EPA together with arachidonic acid when grown at low temperatures. Results from

productivity studies of EPA in various fungal strains under growth conditions of low temperatures indicated that *M. alpina* grows well at low temperatures (5–24 C) and accumulates more than 0.5 g/l of EPA (26.6 mg/g dry cells).

Kenji Soda, also of Kyoto University, talked about a research program in which various microorganisms were tested for their ability to transform oleic acid to ricinoleic acid. A strain of soil bacterium, BMD120, incapable of using oleic acid as a sole carbon source, formed the highest amount of ricinoleic acid from oleic acid in the culture fluid.

S.L. Neidleman of Cetus Corp., the next speaker, said that in many cases, a means could be found to produce a desired lipid from a microbiological source without genetic modification. He added that temperature, directly or indirectly, is one determinant in altering the biosynthesis and distribution of fats and fatty acids. Lipid changes in response to temperature alteration transcend effects on the degree of unsaturation. For example, they may include changes in chain lengths of fatty acids, levels of fatty acid branching and cyclization, and the distribution and relative proportions of members of the glycolipid and phospholipid families.

The alkane-utilizing microorganisms, *Acinetobacter* species HO1-N, synthesize a number of unique lipids when grown at the expense of long-chain alkanes, fatty alcohols, fatty acids and symmetrical long-chain diaklyl ethers, according to W.R. Finnerty of the University of Athens. The metabolism of n-alkanes and fatty alcohols yields wax esters as primary end products. A double mutant of *Acinetobacter* converts fatty acid to short-chain alkane and decanedioic acid. Extension of these studies to the metabolism of long-chain, symmetrical alkoxyalkanes (C14–C20) demonstrated the oxidation of a homologous series of diaklyl ethers to alkoxyacetic acids and dibasic acids.

F. Wagner of Braunschweig Technical University discussed the growth of microorganisms on hydrocarbons or other lipophilic substrates associated with the synthesis of biosurfactants. These metabolites mostly are involved in the mechanism for the initial interaction of the lipophilic substrates with the microbial cell. Biosurfactants are amphiphilic compounds. The hydrophilic part in the molecule can be a carbohydrate, a cyclic peptide or an amino acid. The hydrophobic part contains long-chain fatty acid, hydroxy-fatty acid or α-alkyl-β-hydroxy fatty acid. For the microbial preparation of biosurfactant hydrocarbons, vegetable oils, glycerol or carbohydrates can serve as carbon substrates.

Satoshi Kudo of Yakult Central Institute for Microbiological Research presented a general review of the use of biosurfactants in food featuring recent work on transphosphatidylation of soy lecithin. A new biosurfactant, transphosphatidylated lecithin, in which most of the phosphatidylcholine and phosphatidylethanolamine has been converted to phosphatidylglycerol by phospholipase D, was introduced.

Melanie J. Brown reported that although a wide variety of surfactants is produced by microorganisms and many of these compounds have been identified and characterized, only recently have quantitative assessments of biosurfactant performance for enhanced oil recovery been described. Crude bacterial culture broths can reduce interfacial tension against oil to 10–2 mNm_{-1} and, in the presence of an alcohol cosurfactant, interfacial tensions as low as 10–5 mNm_{-1} have been measured. Such biosurfactants are effective at low concentration and can be made from cheap sources of sugar as substrates.

Shigeo Inoue of Kao Corp. reported that a yeast, *Torulopsis bombicola* KSM-36, produced 10g or more of glycolipids per liter. It has been found that they are a series of derivatives containing, as their backbones, sophorolipid (SL) structures in each of which ω- or ω-1-hydroxy fatty acid has been ω-glucosylated with a sophorose group obtained by bonding two moles of glucose together. Sophorolipid can be produced at a stable rate of about 100g to 150g per liter of a combined carbon source of palm oil and glucose. Alkyl-SL esters were prepared through esterification between a group of long-chain fatty alcohols and SL. P-SL was prepared by subjecting a propylene oxide and SL to addition polymerization. Oleyl-SL and P-SL, being an addition-polymerized product of one mole of SL and about 12 moles of propylene oxide, have specific compatibility to the skin and have found commercial utility as skin moisturizers.

Karl Gander (left) considers a question from a meeting registrant during a session break.

Engineering and scale-up in fat and fatty acid biotransformations

The first of several papers dealing with interesting applications of new bioreactor technology was presented by Jiro Hirano of Nippon Oil & Fats Co.

The highly active lipase from *Candida cylindracea*, widely used in studies for the commercial hydrolysis of oils and fats, was used in this research. The membrane-type bioreactor equipped with many disperser-membranes has been evaluated as a highly efficient and compact reactor. For high-melting fats, the solid phase static process has been evaluated.

Isao Karube of Tokyo Institute of Technology presented the case for the development of biosensors for lipid analysis. In this study, novel biosensors for lipids, including neutral lipids, phosphatidylcholine, cholesterol and free fatty acids, were examined. These methods required no special pretreatment of the sample. Furthermore, measurements were completed within 10 minutes. These sensors

appear to be promising and attractive methods for the routine measurement of lipids.

In the area of membrane bioreactors, the possibility of carrying out biocatalytic conversions between two immiscible phases separated by a membrane makes this technology of special interest to the oil and fat industry where the processing of emulsion is common, according to J. Kloosterman of Unilever. Examples are the hydrolysis of oils and the esterification of fatty acids and glycerol using lipases. Clearly, optimization of a membrane bioreactor (network), in terms of its biocatalytic and economic performance, requires extensive research.

George Abraham of USDA's Southern Regional Research Center focused on the mass transfer occurring in enzymatically catalyzed reactions. The effect of mass transfer limitations is to alter the observed or overall reaction rate which affects the product yield and overall economics.

Mattias Bühler of Henkel, discussing various aspects of continuous processing, stated that reuse of the enzyme seems to be the method of choice. The aim of this investigation was to develop a method for the continuous use of lipases without carrier fixation. This was accomplished by the use of two continuously operating centrifuges. This procedure allowed a kinetically and thermodynamically desirable countercurrent flow of the fat and aqueous phases.

Many of the presentations at the conference alluded to and discussed the economics of lipid biotechnology. The next three papers directly addressed this issue.

N.K.H. Slater of Unilever examined the raw material costs and the various scale-related plant and processing costs for the batch production of microbial lipids. Akio Iwama of Nitto Electric Industrial Co. discussed a feasibility study conducted on introducing membrane separation technology into the refining process for edible oils. He noted it is feasible to degum crude miscella by ultrafiltration using the solvent-resistant polyimide ultrafiltration membrane, and the process is profitable compared to chemical refining.

Ray Dull of Experience Inc. discussed the problems involved with commercializing biotechnologically adapted products in the fats and oils industry. Dull said this requires careful and early attention to a range of production technology, product specification and marketing feasibility issues. Commercial feasibility of biotechnological modified lipids clearly is dependent upon the nature of the lipid produced—whether it is a high-priced material or difficult to obtain.

In the discussion session, speakers said enzymes are being used to modify and produce fats and oils. For instance, Unilever uses immobilized enzymes for interesterification and up to 8,000 tons per year of oil are treated by fat-splitting with immobolized enzymes for soapmaking. Also, phospholipase A-2 is said to be in use to produce a lysopholipid.

Speakers, discussing the obstacle for implementing biotechnology for the fats and oils industry, agreed that economics are the chief factor.

Regulatory aspects

This topic is of crucial importance for the future of biotechnological products. It is important that these products be approached as other new products in their safety testing so there is no mystique attached to them. It has been said that each advance in science follows a new or improved analytical method. In a similar fashion, accurate and reliable methodology must be available to define monitoring and testing protocols used in regulatory aspects of biotechnology.

G.R. Beecher of the Beltsville Human Nutrition Research Center reviewed analytical instrumentation and techniques used to monitor alterations in lipids brought about by biotechnology. Eckhard Scheible, presenting a talk prepared by colleague Rolf Bass of Bundesgesundheitsamt, indicated that for drugs produced by biotechnological techniques, toxicity testing by routine protocols may be inadequate, unpredictive or even impossible to perform. Judgment of safe use of such products may depend, to a larger degree than for chemically synthesized compounds, on the availability of other data, such as that obtained from clinical studies.

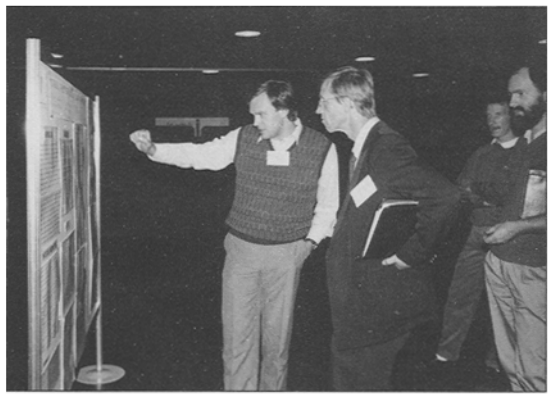
W.E. Parish of Unilever said products of biotechnological processing may be considered in terms of examination for potential hazards for users, protection of the environment from contamination by organisms derived by genetic engineering, and public emotional responses to biotechnological products and processes. Examination for potential hazards requires consideration of the product-forming microorganisms, and whether the product receives genes derived from other organisms. Apart from a screen for mutagenic activity, additional tests follow standard procedures for safe handling and feeding tests, pharmacokinetics and teratology, all supported by full pathology.

P.S. Elias of Bundesforschungsanstalt für Ernahrung reported that the Commission of the European Economic Communities is making efforts to develop a harmonized approach, acceptable to all member states, for the regulation of biotechnological processes and products. A paper prepared by David Kingsbury of the National Science Foundation and read by W. Waldemar Klassen outlined the U.S. government's "Coordinated Framework" providing for interagency coordination for regulating biotechnology, as published in the *Federal Register*. Also, Mitsuyoshi Tamaki of Japan's Ministry of International Trade and Industry presented Japan's policy, as outlined in the Japanese Ministry of International Trade and Industries' "Guidelines for Industrial Applications of Recombinant DNA Technology."

In this session, K.K. Carroll of the University of Western Ontario reviewed data indicating that consumption of high-fat diets has been associated with increased risk from a number of chronic diseases, including cancer and cardiovascular diseases. Also speaking, R. Keith Downey of Agriculture Canada said a major problem area for crop plants is the interphase between patent legislation and plant breeders' rights (PBR), the latter being the proprietary right to a crop variety

as legislated under the International Union for the Protection of New Varieties of Plants (UPOV). Downey, noting that some have advocated replacing PBR with patent law, cautioned that biotechnology must recognize the value and rights associated with protected varieties, which are the vehicle for commercializing patented genes.

During the discussion, session speakers were asked how regulators would view the production of an oil from a traditional plant source that had been modified to contain a nontraditional fatty acid. They agreed it probably would be necessary to submit the oil for a complete toxicity testing protocol, including mutagenicity and teratological studies, since one also must worry about small amounts of other components in fats.



Armin Schmeichel (left) of the Technical University of Braunschweig discusses his poster presentation with Victor Krasnobajew of Givaudan Research Co., Switzerland. Poster sessions featured 41 papers in three days.

Highlights from poster sessions

The following report on the poster sessions was written by Richard F. Wilson, USDA/ARS scientist at North Carolina State University, who chaired the poster session.

Voluntary contributions to the conference were presented in three separate poster sessions. A total of 41 posters were exhibited during the conference by scientists from West Germany, Finland, Israel, Japan, Brazil, France, Yugoslavia, India, The Netherlands, Malaysia, United Kingdom, Spain, Canada and the U.S. These sessions provided a forum for detailed discussion of the state-of-the-art investigations in lipid biotechnology and indepth technical review of information reported in the corresponding plenary sessions. The posters also created a focal point for increased exchange and interaction with conference exhitors.

Although several of the voluntary presentations will be included in the conference proceedings, the following synopsis of the poster presentations highlights the diversity in biotechnological research approaches currently being used in the fats and oils industry. Each poster session focused on a specific theme: biochemical and genetic regulation of lipid synthesis by plants or microorganisms (Session 1), metabolism and mechanisms of lipase-catalyzed reactions (Session 2), and industrial applications of biotechnology for production of biosurfactants and other lipid products (Session 3).

Session 1

The biological potential for production of highly specific lipid compounds was demonstrated by various yeasts, bacteria and alga. In vivo synthesis of corynomycolic acid esters of mono-, di- and trisaccharides was characterized in Athrobacter species. These unique glycolipids may serve as important new types of biosurfactant. The red alga *Porphyridium cruentum* was shown to synthesize glycolipids with high levels of eicosapentaenoic acid and neutral or phospholipids with high levels of arachidonic acid. The level of these lipids was dependent upon temperature, light intensity and nitrogen nutrition. The high yield of these rare acids obtained from controlled environment cultures may provide a viable substitute for fish oils in the control of blood cholesterol levels and other nutrition-related uses.

Other studies demonstrated the production of *trans*-free polyunsaturated fatty acids for margarine by the yeast *Candida scylindracea*, the specific formation of 10-hydroxystearic acid from oleic acid by Norcardia species, and synthesis of steryl-ferulates by lipase preparations.

The molecular genetic and biochemical regulation of oil composition and quality was considered in a second group of papers in Session 1. These papers contained first reports on the gene sequence of a δ-9-desaturase from *Saccharomyces cerevisiae*, which catalyzed desaturation of stearate to oleate and palmitate to palmitoleate. Sequences for lipoxygenase LOX-1 and LOX-3 genes demonstrated a high degree of homology between soybean and tobacco cDNA. This work also identified probable errors in the identification of the LOX-1 gene previously published by other laboratories.

Finally, triacylglycerol (TG) synthesis and regulation of TG molecular species composition were addressed in studies with the oleaginous yeast *Apiotriuchum curvatum*, cocoa beans, sunflower, soybean and rapeseeed. These studies provided evidence that TG synthesis and oleosome formation was not associated with the endoplasmic reticulum, that the high oleic trait in sunflower was determined by three genes, and that substrate utilization by diacylglycerol acyltransferase was a final determining factor in the production of TG molecular species in soybeans selected for altered fatty acid composition. Collectively, all of the papers given in this session demonstrated the importance of exploiting natural genetic variability among organisms in biotechnological research for the fats and oils industry.

Session 2

The function of the enzymes classified as lipase usually is associated with catalysis of TG hydrolysis in nearly all biological tissues. Research with rapeseed reported two different lipase activities localized in oleosomes (18.5 kD protein) and microsomes (56 kD protein), respectively. During germination of rapeseed, activities of the 18.5 kD lipase were high initially, but TG hydrolysis appeared to be related to later developmental increases in the activity of the larger peptide. A lipase enzyme-linked immunosorbent assay (ELISA) method was developed for the purification of oilseed lipases. However, the more significant biotechnological value of lipases may be found in the ability of these enzymes to synthesize or transesterify TG with different fatty acids and a variety of other compounds such as fluorinated hydrocarbons.

Several methods were described for elucidation of the mode of lipase action and enzyme kinetics. The use of lipase to produce value-added products from sal, mango and mowrah fat by enrichment of TG with palmitic or stearic acids also was described. This latter work could yield significant economic benefit to the fats and oils industry in underdeveloped nations.

Session 3

Synthesis of novel lipid compounds by organisms in vivo or via specialized enzyme catalysis constitute biotechnolgical opportunities for the industrial sector. However, the successful application of basic knowledge to achieve some tangible, hopefully profitable, product is dependent upon many factors. Several of these factors were the subject of the investigations presented in this session.

Excluding industrial production costs, characteristics of the end-product should have more than theoretical value. Biosurfactants resulting from biotechnology may exceed that criterion. Microbial production of sophorose lipids, rhamnolipids and polycarboxylic molecules such as spiculisporic acid were examples of the advances made in biosurfactant product development that may have sigificant impact on conventional processes and current product use.

Other engineering problems facing the industrialization of biotechnological research include the development of efficient bioreactors for immobilized lipase applications. Both batch and continuous systems were compared with respect to removal of fatty acid from the oil without excessive oil loss, optimization of flow rates and reaction performance, selection of membranes, destruction of foams inherent to these processes, and bringing these model systems up to an acceptable industrial scale of production.

Solutions to these problems must be found. The reports in this session represented a significant body of information that has advanced practical efforts to achieve those goals.

Biotechnology and the public

The American public generally favors placing strict limitations and establishing international agreements on genetic engineering, according to "The Novo Report: American Attitudes and Beliefs About Genetic Engineering" conducted for Novo Industri A/S by Research & Forecasts Inc.

More than 1,000 Americans and select science and ethics experts were polled for the study. However, approximately two in five polled had not heard of genetic engineering. Consequently, these individuals were disqualified from participating in the remainder of the study.

According to the report, 70% of respondents familiar with genetic engineering agreed that strict limitations should be imposed on the kinds of genetic engineering research scientists are permitted to conduct. Also, the report said the public believes scientists, ethicists and government should work together to determine regulations. Those polled cited the American Medical Association, genetic engineering scientists, the federal government and persons concerned with social ethics as the ones to be involved in regulating genetic engineering.

"The American public realizes the complicated issues involved in genetic engineering and is wise to look for guidance from experts in ethics, science and government," Ulrik Lassen, Novo Industri's chief science officer, said.

Two out of three Americans polled support the establishment of international guidelines for genetic engineering research. Three out of four favoring an international agreement felt it should be enforced by an international group of scientists rather than the United Nations or an international group of statesmen.

Exhibit highlights

The exhibit accompanying the World Conference on Biotechnology for the Fats and Oils Industry included 26 booths staffed by representatives from 20 companies. Some featured products and services specifically geared for biotechnology; others offered more mainstream processing equipment. Here is a look at the highlights of the exhibit.

- **Amandus Kahl Nachf. GmbH & Co.** featured an expander which the company has designed and manufactured upon licensing from Heinz Schumacher. The expander is designed to improve soybean and sunflowerseed extraction.
- **Atlas Industries** offered a dry condensing plant system for producing vacuums in the 1 torr-10 torr range, designed to cut energy usage and pollution production. Units have been installed in facilities in West Germany and Scandanavia, where pollution prevention is stressed and energy sources are expensive.
- **ATT-Verfahrenstechnik** featured its ATT-process for convert-

Visitors to Extraktionstechnik GmbH's booth (above) learn about the company's equipment and latest improvements. In the photo at left, Westfalia's latest centrifuge is a main attraction.

ing vegetable oils to diesel fuels. Company representatives said pilot plants will be established in Europe during 1988. The process can be used both in small and large-scale operations, of 5–400 metric tons per day.

• **Bruker Analytische Messtechnik GmbH** promoted its instruments using solid fat index for application to the food industry and for oil and moisture determination in oilseeds. The company is seeking approval as an official method in Europe for this latter use.

• **Caffaro S.p.A.** offered a variety of bleaching earths for fats and oils applications.

• **Coesfeld GmbH** promoted its viscometers and other testing equipment.

• **Extraktionstechnik GmbH** said it is supplying equipment for the first oilseed processing plant in Austria, a rapeseed/sunflowerseed facility slated to go onstream in 1988. It also is supplying a fully computerized oilseed processing plant, including refining, for the Heilongjang Province of China, to be in operation by the end of the year. Stressed at the exhibit were environmental improvements in the company's effluent-free extraction plants, physical refiners/deodorizers and continuous deodorizers.

• **Franz Kirchfeld GmbH KG** and **S.A. Fractionnement Tirtiaux** exhibited together. A special feature was Tirtiaux's equipment to produce spreadable and low-calorie butters, products that are experiencing growing demand.

• **Krupp Industrietechnik GmbH** featured its Elcrack process, which combines opening animal cells using a high-voltage alternating electrical field and subsequent mechanical solid-liquid separation. This continuous process can be used in processing fish, animal and fish offals and rendered fats, and offers low process temperature and energy consumption, according to the company.

• **Krüss GmbH**, which in 1983 introduced electrical cell fusion instruments for biotechnological applications, promoted its new third-generation processor-controlled electro-cell fusion apparatus used in genetic engineering for plant, mammalian and yeast bacteria cells.

• **Hewin International Inc.** fefeatured technical studies it has conducted for the industry. These included reports on Biotechnology in Animal Health and Nutrition, Biotechnology in the Manufacture of Specialty and Commodity Chemicals, Industrial and Institutional Surfactants, New Applications of Microbiology to Chemical Processing, The Use of Chemicals in the Fish Farming Industry in North America and Europe, and The Impact of Biotechnology on the Oils and Fats Industry.

• **Heinz Schumacher V.D.I.**, known for developing the desolventizer-toaster-dryer cooler (DTDC), promoted a process for fullfat soymeal for feeding purposes, in which final products are differentiated by quality based on the content of very high or very low protein dispersability index (PDI) or nitrogen solubility index (NSI).

• **Körting Hannover AG** featured its new CCF system, which combines ejectors, condensers and flash cooling with a refrigerator unit, to use in the edible oil industry. Company representatives said this has been used in a test plant in West Germany during 1987 and will be installed in a large plant in Hamburg in early 1988.

• **Novo Industri A/S** promoted its immobolized lipases for ester synthesis. A company representative spoke on this research area during the technical sessions at the conference.

• **Sanki Engineering Ltd.**, a new exhibitor, introduced its large-scale separation process for the fats and oils industry to Europe at this exhibit. The firm has been conducting cooperative research with the University of Lausanne, Switzerland.

• **Schmidding-Werke**, a new exhibitor, promoted its falling film apparatus for the industry.

• **Süd Chemie AG** promoted its range of products for possible application to the biotechnology industry, such as its bentonite as a carrier for enzymes.

• **Tintometer GmbH** representatives, demonstrating a new oil discard tester, said they were "testing the market" on this simple equipment designed for rapid assessment of the condition of frying fats in use at restaurants or fast food establishments.

• **Westfalia Separator AG** featured its new centrifuge for refining, degumming and fractionation of edible fats and oils developed to reduce noise. It includes a hood to reduce noise to 76 decibels. The unit can be used to handle up to 300 metric tons per day. Company representatives said three of the new units were commissioned in a plant in The Netherlands during September. Regulatory pressure from government for noise abatement has prompted the development of this equipment.