Food Microbiology

Volume II
New and Emerging Technologies

Editor
Thomas J. Montville, Ph.D.
Associate Professor
Department of Food Science
Cook College, New Jersey Agricultural
Experiment Station
Rutgers, The State University
of New Jersey
New Brunswick, New Jersey

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging-in-Publication Data

Food microbiology.

Contents: v. 1. Concepts in physiology and metabolism — v. 2. New and emerging technologies.
Includes bibliographies and index.
"This book is publication no. C-10112-2-85 of the New Jersey Agricultural Experiment Station" — Pref.
1. Food—Microbiology. I., Montville, Thomas J.
[DNLM: 1. Food Microbiology. QW 85 F6852]
QR115.F643 1986 576'.163 86-24461
ISBN 0-8493-6474-4 (set)
ISBN 0-8493-6478-7 (v. 1)
ISBN 0-8493-6479-5 (v. 2)

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-6474-4 (set)
International Standard Book Number 0-8493-6478-7 (v. 1)
International Standard Book Number 0-8493-6479-5 (v. 2)

Library of Congress Card Number 86-24461
Printed in the United States

THE EDITOR

Thomas J. Montville, Ph.D., is Associate Professor of Food and Fermentation Microbiology at Cook College, Rutgers — the State University of New Jersey.

Dr. Montville received his B.S. degree from Rutgers University in 1975 and was awarded his Ph.D. at the Massachusetts Institute of Technology in 1979. He then spent five years in Philadelphia as a Senior Research Microbiologist at the U.S. Department of Agriculture's Eastern Regional Research Center prior to taking up his present post in 1984.

The author of 25 research papers and numerous abstracts in the areas of oral, food safety, and fermentation microbiology, Dr. Montville is a member of the American Society for Microbiology, the Society for Applied Bacteriology, the Society for Industrial Microbiology, the Institute of Food Technologists, and the American Association for the Advancement of Science. He has served on the editorial boards of *Applied and Environmental Microbiology* and the *Journal of Food Protection*, and as an editorial consultant for several publishers.

Dr. Montville's main areas of research interest are the bioregulatory mechanisms that govern the synthesis of flavor compounds by lactobacilli, applications of biotechnology to food science, and the influence of extrinsic factors on *Clostridium botulinum* toxigenesis.

CONTRIBUTORS

Volume I

Carl B. Anderson, Ph.D.
Senior Microbiologist
Department of Microbiology
Del Monte Corp.
Walnut Creek, California

Jiann-Shinn Chen, Ph.D.
Associate Professor of Microbiology
Department of Anaerobic Microbiology
Virginia Polytechnic Institute & State University
Blacksburg, Virginia

Bonita Glatz, Ph.D.
Professor
Department of Food Technology
Iowa State University
Ames, Iowa

David Gombas, Ph.D.
Group Research Scientist
Campbell Institute for Research & Technology
Camden, New Jersey

Peter C. Maloney, Ph.D.
Assoicate Professor
Department of Physiology
The Johns Hopkins University School of Medicine
Baltimore, Maryland

Thomas J. Montville, Ph.D.
Associate Professor
Department of Food Science
Cook College, New Jersey Agricultural Experiment Station
Rutgers, The State University of New Jersey
New Brunswick, New Jersey

Lloyd Witter, Ph.D.
Professor of Food Microbiology
Department of Food Science
University of Illinois
Urbana, Illinois

Volume II

Michael Cancro, Ph.D.
Associate Professor
Immunobiology Unit
Department of Pathology
University of Pennsylvania
Philadelphia, Pennsylvania

Ruth Firstenberg-Eden, Ph.D.
Microbiology Research Manager
Research and Development Department
BACTOMATIC
Princeton, New Jersey

Michael J. Haas, Ph.D.
Lead Scientist
Molecular Genetics Group
U.S. Department of Agriculture
Philadelphia, Pennsylvania

Laura Jane Kienker, M.S.
Research Associate
Department of Pathology
University of Pennsylvania
Philadelphia, Pennsylvania

Thomas J. Montville, Ph.D.
Assoicate Professor
Department of Food Science
Cook College, New Jersey Agricultural Experiment Station
Rutgers, The State University of New Jersey
New Brunswick, New Jersey

Anthony N. Sharpe, Ph.D., F.R.C.S.
Head, Microbiology Automation Section
Bureau of Microbial Hazards
Health Protection Branch
Health and Welfare Canada
Ottawa, Ontario, Canada

Joseph Zindulis, Ph.D.
Manager, Quality Assurance & Applied Research
Research and Development of Microbiology
BACTOMATIC
Princeton, New Jersey

TABLE OF CONTENTS

Volume I

Volume II

Chapter 1

POTENTIALS AND IMPEDIMENTS IN AUTOMATED FOOD MICROBIOLOGY*

A. N. Sharpe**

TABLE OF CONTENTS

* Received in final form May 1985.

** The author's views are not necessarily those of the Health Protection Branch.

I. INTRODUCTION

From time to time typical scenarios are discussed in every boardroom and coffee corner across the food industry. They entertain such probabilities (or certainties) as rising energy costs for traditional food processes, mounting demand to eliminate additives, the "quality" of gentler processes, "cheaper" bulk packaging, "natural", "health" and "ethnic" foods, increasing pressure by consumer organizations for tougher legislation or introduction of legislated standards, the imminent flowering of single cell and other biotechnologies, and so on. In each case the indications will be of increased preoccupation with the microbiological quality of food, by consumers, by regulatory agencies, and by food manufacturers.

There is good reason for this concern, of course, because foodborne illness abounds and it costs enormously. While the official data reported by health agencies do not, of themselves, look too bad, they are believed to be even less than the tip of the iceberg. In 1980 for example, Hauschild and Bryan[1] estimated the prevalence of human salmonellosis in Canada and the U.S. to be about 150,000 and 740,000 cases a year, respectively, or 30 times greater than official figures suggest.

Investment in microbiological quality assurance may never have stamped the fortune of a food company, but ignoring it has certainly ruined some and will ruin more in years to come. Likewise, though a marriage of microbes into the fertility of foodstuffs may not have caused the birth or miscarriage of many political careers it is a capricious source of embarrassment. So a great deal of corporate or taxpayer's money undoubtedly will be spent over the next few years to ensure acceptable microbiological quality in food. There may be more surveillance, more in-house laboratories, improved sanitation programs, plant changes, personnel education, legislation, actions, recalls and the like. Basic to all these activities will be, as now, a means of actually determining quality, that is, microbiological analysis.

Thus, the importance of microbiological analysis will increase, and the constraints of time, cost, and throughput will guarantee the proliferation of automated microbiological instruments. Or will they? In fact, can we even be sure of the increased importance of microbiological analysis?

Further on I shall discuss the possibility that the value of food microbiological analysis — and, therefore, interest in its automation — could wane. Would-be purveyors of microbiological automata have always had a hard time, but it is an intriguing thought that this bastion of 19th-century science might never be induced to decorate itself adequately with microelectronics and might remain essentially what it is today, quaintly anachronistic, yet with an analytical capability captivating scientists from more technologically advanced fields when they (too rarely) take the time to study it.

II. THE POTENTIAL FOR AUTOMATED FOOD MICROBIOLOGY

Not the least question to be asked when discussing the potential for automated food microbiology is — is there any potential at all? That is, will the introduction of instruments capable of carrying out analyses faster, in greater number, more accurately, more precisely, at less cost — and all the other accepted attributes of automation — noticeably reduce the incidence of food poisoning? At the national level the answer is probably *no*.

The reason is that, while we are all becoming more sensitive to the notion of quality in food, the general education of consumers in the basic microbiological necessities of housekeeping remains — and appears to remain — woefully inadequate. These days the fundamental microbiological problem of food rests not in its manufacture and inspection but with the consumer. For example. Todd[2] estimates that mishandling by consumers or caterers may cause 97% of illnesses proven due to foodborne microbes. Even a dramatic improvement — halving say the 3% of incidents traceable to the manufacture and distribution of foods,

and regardless of the cost of achieving it — might not be sufficient to produce a significant downturn in the smudge of national food-poisoning statistics.

Nationally then, the stimulus for automated methods is weak. However, in moments of discomfort most consumers will automatically assign any blame to the food manufacturer and any laxity to the regulatory agency, and the news media will almost certainly fail to straighten things out. So it is best not to get involved, since the prognosis for any food company implicated by one of those 3% of cases is more than gloomy enough for the majority to pursue a keen interest in microbiological analysis, in better ways of doing it and, therefore, in automation. This interest is reinforced by the technological changes now occurring in the food industry. Increased microbiological testing is indicated as food processing and storage methods change, for new microbiological problems are constantly appearing while old ones die slowly.

As with the food manufacturing industry, interest in automation by regulatory and public health agencies is generally strong, for these bodies are caught between opposed forces. On one hand, both increasing international movement of goods and the new processing technologies necessitate an increased level of routine monitoring. In addition, there is a growing need to test routinely for organisms previously considered "unusual". Unfortunately, most public agencies also find themselves constantly squeezed by political pressures to reduce the size of government departments. Most of them periodically restructure work schedules, duties, etc. in an effort to cope, but massive automation is generally seen as essential at some future point.

While the goals and problems of the dairy industry generally differ little from those of other areas of food microbiology, the control of raw and pasteurized milks presents a unique subset of problems. Milk is relatively easy to disperse, sample, and plate compared with other foods. The microbiological goals of the agricultural agencies or marketing boards (at least, in regard to payment-by-quality and process control) are relatively easy to accomplish. The very large number of routine tests required at this stage, the ease with which the cost effectiveness of instrumentation can be calculated, and the simplifed engineering problems combine to make this an attractive area for automation. A number of automated methods, for example, for diluting and plating samples, counting colonies, microcolonies or somatic cells, and estimating phosphatase or pyruvate already are used successfully and I shall not pursue these further.

III. POSSIBLE AVENUES OF AUTOMATION

At this point, having ascertained that there may actually be potential for automated food microbiology, let us look briefly at the directions it might take. I shall then follow the various threads through their anticipated merits, trying to suggest a suitable rationale (if, indeed one can be made) for the slowness of progress. There seem to be four main possibilities, only three of which are actually directed towards microbiological analysis:

1. Automation of conventional enumerative microbiology, or the presence/absence tests, which are conceptually the same. Being the most direct extension of conventional microbiology this approach entails the minimum conceptual leap. It would probably be the most immediately acceptable choice to serious purchasers, if satisfactory automata were available.
2. Automation of "metabolism" based techniques in general. These, as I discuss further on, do not reveal good "counts", but rather, some measure of the microbial potency or virility. One might think this would be an excellent thing to know about a sample. Nothwithstanding, metabolic techniques are currently used mainly as substitute ways of doing plate counts, their data are therefore suspect and consequently are marginally

acceptable. Their improved acceptability and proliferation awaits the relegation of plate counts as food quality determinants.

3. A subset of these "metabolic" measurements provides data only on those microbial manifestations pertinent to human physiological or psychological responses. That is, on the physical, chemical, and biochemical changes responsible for the organoleptic, toxic, or infective properties of a food. It is possible, in principle at least,[3,4] to adapt such measurements to actually predicting the effects individual food samples will have on consumers, which may eventually prove the most profitable avenue in microbiological analysis. Unfortunately, this subset does not include several of the currently most promising metabolism-based techniques and, therefore, sets us back a little.
4. Finally, the biggest advances in quality assurance prior to purchase will probably result from extensive integration of electronic sensing and slave systems into computerized manufacturing and distribution processes. In such processes, all microbiologically relevant parameters (temperature, time, pH, Aw, etc.) would be continuously monitored and controlled to ensure optimum quality. Building microbiological quality into food, through Impeccable Manufacturing Practice, might drastically reduce the need for routine analysis and, therefore, make further efforts to provide analytical automata superfluous.

IV. THE PROBLEMS

Is there any reason to believe that massive introduction of automated instruments is not just around the corner? At first sight, survival of the manual test might seem impossible. Neither speed nor precision have ever been hallmarks of microbiological analysis. Answers (always too few of them) are days, sometimes a week or more coming in, and their significance is not always obvious. The benefits of automation seem overwhelming at least until the moment the instrument salesman walks through the door.

At this point it may be instructive to examine the analytical problems and capabilities of food microbiology against those more technologically advanced analytical sciences that are sometimes held up for comparison. With little exception, food microbiology is a science of trace materials; hardly ever do the weight fractions of the microbes under study exceed 1 ppm in food. So it is not unreasonable to compare food microbiology with pesticide and dioxin analysis, which currently enjoy much glamour, and for which much instrumentation and funding seem to be available.

Situation — The chlorinated pesticides (DDT, chlordane, etc) comprise a closely related group of compounds currently detectable at about 0.5 ppb, though with fairly large confidence limits in this level. The concentration ranges are relatively small — perhaps a factor of 100. The dioxins are similarly related. They can be detected at levels down to 1 to 5 parts per trillion, or 0.5×10^{-12} 1g. In fish or fatty products the analysis may take 4 to 5 days, and the necessary chromatographic equipment and mass spectrometer cost $0.5 million (1986 dollars).

Situation — A *Salmonella* cell weighs in at around 10^{-13} g or, say, 10^{-16} of a chicken. Anticipating but ignoring the shower of qualifiers that may be raised by more experienced microbiologists, I will state that such a cell is eminently and easily detectable on the chicken, by routine bacteriological techniques requiring no more than a few 10c Petri dishes and flasks of media, plus an incubator costing a few hundred dollars. The confidence limits are rather large at this level. By older techniques the analysis may take 4 to 5 days; with some newer developments it may take 1 day.

This capability comparison may be taken one step further. It is likely that at least 99% of the *Salmonella* cell is chemically indistinguishable from similar material in the chicken. In terms of specific chemicals, therefore, the traditional manual *Salmonella* analysis is capable of detecting one part in 10^{18} of food.

Of course, the *Salmonella* analysis is usually carried out simply as a presence/absence test, but many other microbiological analyses are routinely geared to handling concentration ranges of at least 1,000,000:1. And even within the dozen or so widely different analyses the microbiologist may be routinely called upon to do, phenotypic variations may rival or exceed those within the dioxins.

Practictioners of the "more advanced" sciences might bow their heads; this maligned traditional microbiology has formidable capability. More to the point, since despite the microbiologist's many manipulations it is the microorganisms that do the real work of making their tiny signal detectable, one wonders just what advantages automation is going to provide.

The conventional microbiology has a further asset. Although many laboratories routinely process samples for one or two analyses throughout the day (e.g., TVC, *Escherichia coli*), certain types of analysis may be needed only intermittently. But almost invariably, instruments which have to manipulate liquids, such as are indicated for many microbiological analyses, do not take kindly to intermittent operation. And food microbiology cannot yet hope to emulate, say, clinical biochemistry, where it is acceptable for the analyzer to automatically carry out every conceivable analysis, be it necessary or not. In contrast, the microbiologist with a small stock of refrigerated media is ready for almost any demand, as it arises during the day.

Moreover, as long as the only acceptable answers are those in terms of numbers of organisms, or numbers of colony forming units, there is little substitute for the plate count (or its conceptual equivalents). There is no denying that analyses based on metabolic expressions of microorganisms do not correlate prettily with plate counts. Leaving aside for the moment the very fundamental question of whether anyone should even want data that correlate with plate counts, the facts are

1. That if we want automated instruments to yield convincing plate counts these instruments will have to actually carry out plate counts, with all the dispensing, mixing, and slopping that it entails. They are, therefore, so expensive to develop that not a single one graces the marketplace, and probably would not be affordable if it did.
2. Instruments which carry out metabolic measurements are so much more feasible that they are actually available. However, their data correlate only fitfully with plate counts. Some reported "correlations" suggest to me more "tongue-in-cheek-author" than "hand-in-glove-data", and I am sure I am not the only one to perceive data in this way. This does little to improve the desirability of metabolism-based techniques, if one is committed to counts. It is possible, as the electrical impedance people demonstrate, to use whatever correlation can be obtained to screen out the very bad samples and the very good ones, then subject the remaining samples to the manual counting procedure. This, at least, gives some confidence to the count data, and saves time on a proportion of the samples.

It is against the innate analytical capability of manual food microbiology, and its secure establishment, that the instrument salesman must make his pitch. In terms of analytical performance, the instruments thus far offered to microbiologists do not show a dramatic superiority. And though food poisoning may be a bigger, certainly an older, national problem than dioxin contamination, it does not have the support of public awareness. Few microbiologists could convince their superiors that they needed a $0.5 million analyzer to detect *E. coli*, another to detect *Staphylococcus aureus*, another to detect *Clostridium perfringens*, and so on.

This is a good place to discuss the food microbiologists's poor bargaining position, and it is all connected with the elusiveness and ephemerality of microbial counts. The data are not "hard" in the way that many other types are perceived to be. For example, the chemist

with his atomic absorption spectrometer might detect mercury in fish. The data are unequivocal, he would expect much the same answer by some other analytical method. Not so the microbiologist analyzing meats for *Staphylococcus aureus*, which will not be so uniformly distributed anyway, and who even changes brands of medium at his peril. Moreover, the analyzed level of mercury can be related instantly to the consumer's anticipated dosage. For, unlike microbial contamination, mercury does not multiply while the consumer is in bed.

Thus, the quality assurance microbiologist, unlike his chemist neighbor is not generally able to use his data to predict confidently the prognosis of a particular batch of food for the consumer or the company. Because of this the industrial microbiologist's views are often subordinate to those of the plant manager, his findings may be considered but ignored.

A visitor peeping inside the microbiology lab might quickly sympathize. Where — among those torrid annexes spewing plates of simmering media, the ritual cleansings by flame, prescribed poising of bottle caps between pinky and palm, endless stacks of Petri dishes (all streaked and inverted), bags of mangled, scalded remains, imposing Latin names — are the hard data the plant manager needed last Thursday, data guaranteeing that the product will reach its stamped date without bringing disrepute upon them all? The chemist next door, switching off his spectrometers, tells him the product has acceptable levels of water, fat, lead, or whatever, and he feels confident about these. Though many managements view their microbiologist's craft with respect, others hold it with a suspicion usually afforded the darker and less reputable arts.

The corporate microbiologist's duty is to steer the company profitably through the complex mesh of costs vs. quality. Whatever control may be exercised over materials and processing, ultimately and unquantifiably the rather negative measure of his or her success is the ability of the product to remain on sale without damaging repercussions.

In an ideal world it might be agreed that the microbiologist is free to use whatever manner of tests best fit this end. In reality, of course, no test gives complete assurance; neither do variants of a test correlate perfectly with one another. Thus, factors other than the straightforward microbiological performance and cost of a test tend to dictate which one is used. In particular, since even products of the best manufacturing practice can give trouble the respectability of the lab can at any moment be called into account and there is a decided comfort to be drawn from laboratory records which show negative results, not by some potentially superior method (automated or not), but by the official procedure.

Moreover, there is a distinct argument in favor of status quo on methods, not only as far as automation is concerned. For the introduction of a markedly superior test, for example, one which detects a pathogen at a lower level, or simply in a larger number of samples, may unfavorably prejudice the price and nutritional or organoleptic properties of the product if it has to receive unduly severe processing in order to achieve a negative result. Nor can a regulatory body use or recommend such a test when a statutory limit exists for a product. These factors must be weighed against the apparent cost and other savings of any new analysis.

A. The Perceptual Problem

Before reaching the nub of this chapter — the credibility problem facing novel microbiological techniques — I want to delve briefly into a much more controversial area. There are no hard facts here, but it may be useful to carry some of the argument into the succeeding sections if it does bear, as I believe, on the acceptability of automated techniques. I have written much at times[3,4] (and been frequently scolded for it) on the possibility that an emotional crutch supports our retention of enumerative microbiology. That is, on providing some explanation for our unrelenting grasp on quantifying food quality by means of microbial numbers, when it is manifest that counts do not:

1. Usually even accurately represent the number of cells of the species analyzed for
2. Relate to any instant, other than the instant of sampling in the life of a very changeable food
3. Indicate the ability of even those organisms "counted" to multiply in the food between the instant of sampling and the instant of consumption
4. Indicate the lustiness of each organism in contributing to spoilage, toxicity, etc. before consumption
5. Indicate the effect of synergism and inhibition by other components of the microbial flora or of compositional variations in the food on factors 3 and 4 above, before consumption
6. In particular, indicate how the magnitude of these combined and accumulated degrading effects on factors 3 to 5 above, will compare with the consumer's probable threshold of response at the instant the food is consumed.

Indeed, how can we explain this when by simply incubating the food and measuring the development of those physical, chemical, or biochemical parameters to which we know the consumer responds, we might provide an answer encompassing all of these six factors? That would, in fact, tell us something real about the sample. We have this compulsion for counting microbes when it is not generally they that spoil food or make us ill but the physical, chemical, and biochemical manifestations of their existence or multiplication. Most readers would query this in the case of infectious pathogens, but I shall not take up the argument here.

I have described elsewhere[3,4] the principles of measurements that would permit us to predict the effect of foods on consumers. To a large extent, the instrumentation needed for this kind of measurement already exists. In contrast, with slight exception, there is little practical automation for doing counts. If the plate count (this analysis which tells us so little in real terms) is so hard to automate, whereas most physical, chemical, and biochemical analyses (which generally tell us something more meaningful) are so easy to automate, it seems inconceivable that we can still, in 1986, base most of our assessment of food quality on it. Something must lend to the count an aura of credibility or desirability that other measurements lack.

I must speak only for myself here, in trying to explain this. For example, why do I, as a would-be "automater" of microbiological analysis (and who castigates to the world the value of the count) still turn to it instinctively in order to "get the feel" of a sample? Why am I more comfortable with it than with any other datum? My explanation comes solely from introspection and it may not satisfy others. I admit to being a very human animal. For me, the credibility of a count is bolstered by very primitive events:

1. My conditioning during infancy, long before I studied microbiology, to associate bacteria (germs) with danger. It never died, but simply lurks beneath my labcoat.
2. My natural tendency, reinforced by education, to relate to concrete concepts such as "individuals", "people", "creatures", "armies", etc., before more abstract ones such as "concentration", or "$ohm^{-1}\ hr^{-1}$".
3. The disposition of counting procedures to present microbial contamination in terms that excite these primitives. For example, as "cells" (in DMC), or "colony forming units" (read "cells") from which I can instantly (if mistakenly) visualize the strength of the enemy or the enormity of the peril.

Other types of analysis simply do not yield data that excite me as fundamentally as this. So, my apperception of danger is more real for knowing my sandwich contains 100,000/g of fecal organisms, than for knowing it contains 0.02 units of glutamate dehydrogenase

activity. Simply because the image of a little sterculean army makes a much greater impression on my subconscious than does an arbitrary biochemical activity value. I am an automation researcher and avowed opponent of plate counts; if *I* have studied ATP, impedance, dye reduction, catalase and other techniques, yet do not feel comfortable with their data, then what attractions can they hold for other microbiologists? But this explanation may not be for you.

B. The Credibility Problem

With perhaps two exceptions, automated instruments based on the conventional enumerative technique have not been developed to a stage where they could be offered with any real chance of penetrating the food microbiology market. The exceptions are the spiral plating/laser counting and hydrophobic grid-membrane filter (HGMF) systems. Both rely on an ingenious sidestepping of the need to make sequential dilutions which is, perhaps, the worst of the engineering problems to be overcome in providing enumerating instruments at realistic cost.

The Spiral Plater, which was the first of these systems marketed, has made significant inroads in situations where relatively high bacterial counts permit analyses from its relatively small sample volume.[5-8] The performance of the plater has earned it acceptance by the Association of Official Analytical Chemists (AOAC) for official actions with total viable count (TVC). The laser-based scanning/counting system has been less favorably received by food microbiologists. However, the new computerized product of the company is claimed to be able to distinguish those areas on the Petri dish suitable for counting from those degraded by spreading, confluence, etc. and therefore produce a more accurate count. I would expect that, if the claim is substantiated, the spiral plating method will be used in many more food microbiology laboratories, for TVC and other suitable counts.

The HGMF is a more recent arrival and its acceptance is still less certain than with the Spiral Plater. Since many foods may be concentrated on the filter it permits counts to be made at lower levels, making it applicable to analyses other than TVC. For example, it has AOAC acceptance for the coliforms and *E. coli*, and the methodology is being extended to all of the common organisms.[9-15] Thus, it is poised to accommodate all analyses a lab might make, in a single system. Unfortunately, although automated counting of the HGMF grid has been demonstrated in research papers,[9-11] a scanner is not yet commercially available and this may retard its acceptance as a complete laboratory system.

Although neither can be thought of as totally automated — in the sense of one's being able to put in a sample and forget about it until the answer pops out — both the spiral plating and HGMF systems are approaches to automation of the traditional count of colony forming units. Thus, although both may ultimately be seen as stricken with all the drawbacks accompanying food quality assessment by counting CFU, they do currently enjoy the great advantage of being perceived simply as small modifications to the traditional and, therefore, as likely to yield closely related and believable data.

The techniques with real potential in automated analytical food microbiology in the near future are those based on measurement of the physical, chemical, or biochemical manifestations of microorganisms. Such techniques are generally referred to as "metabolism-based" although the term seems to me to be a little stretched at times. Scores of metabolism-based techniques have been described over the years. Dye reduction, light scattering, release of radioactive carbon dioxide from labeled substrates, enzyme activity, adenosine triphosphate or trimethylamine content, and electrical impedance measurements are just a few.

To the nonmicrobiologist, metabolism-based techniques possess two overwhelming advantages over plate counts: (1) the analyte is (almost axiomatically) a single and reproducible property of matter, for which an automated measurement technique often already exists or can be developed, and (2) unlike the plate count it can reasonably be argued that certain

metabolic measurements actually bear some relation to the probability of the organisms causing spoilage or disease.

It is a fact that people who work intimately with microorganisms tend to be less sanguine about metabolism-based techniques. My own perplexity is described under "The Perceptual Problem" (Section IV.A). Graphs and statistics notwithstanding, evaluating metabolic data is a very personal thing and I present an argument below, though I do not necessarily claim it as my *modus vivendi.*

Before going further we should distinguish between "metabolism-based" measurements made at a single instant in the life of a food, and those where the analysis depends on incubation of the sample to provide the datum. The former (for example, adenosinetriphosphate, pyruvate, trimethylamine, ammonia, pH, Eh) must be classed as little better than plate counts in providing information on what the organisms are capable of. The reader is referred to the six deficiencies of counts described in Section IV.A and should simply substitute the measured property for "count" in this list.

In fact, as I hope to make clear below, it is not these measured properties themselves that I consider of such little value. They could form the basis of very useful tests of microbial quality. Their principle handicap is the existence of analytical techniques sensitive enough to detect them at levels corresponding to the levels of microbes normally found in foods. As a consequence, that is how they are employed, in making single measurements on foods, in hopes of relating the data to plate counts.

There are several considerably more useful metabolism-based techniques. These are based on incubating samples for whatever period is required for the analyte to grow from an insignificant signal to a specified or measurable value. I believe that the originators of most of these techniques perceived their relative insensitivity as a problem to be eventually overcome. The truth is that in their relative insensitivity lies their strength, since one is forced to rely on a little incubation to detect the analyte. An incubation during which, if one wishes to perceive it in the right light, the unique combination of organisms existing in the specimen can express to the microbiologist its particular ability to affect the quality of the specimen. No datum pertaining only to a single instant in the life of a food can do this. (Notwithstanding that most people would regard the plate count as the epitomy of techniques requiring incubation, the fact is that a count pertains only to the instant the sample was plated out. Therefore, I do not class it as an incubation technique.)

For example, though few were prepared to admit it, the methylene blue reduction test for milk was, in the days before refrigeration, a much better indicator of milk shelf-life than was the plate count.[16] It was more meaningful simply because it gave some measure of microbial performance. However crudely this might have correlated with, say, souring or ropiness, the combined multiplicative, interactive and respiratory activities of the total milk flora that were needed to decolorize methylene blue during the test said something about the sample that a plate count was quite incapable of.

These days the mesophilic flora have gone from milk and the methylene blue test is no more. In an effort to save its attractions diehards replaced it by resazurin, a reagent so delicate it is turned in a trice. So delicate, in fact, that leukocytes or other nuisances reduce it readily and prolonged incubations are not practicable. Compared with the methylene blue test, the resazurin test relates more to an instant in time and thereby assumes the deficiencies of counts.

The attractive metabolism-based test of today is electrical impedance. It was Nature's gift to the electronic age. Impedance cells can be sampled at different frequencies to provide conductive or capacitative signals. Impedance meters yield an electronic signal which can be interfaced, multiplexed, transcoded, digitized, stored, plotted, recalled, compared, and otherwise processed effortlessly by computers for hours on end. Unlike most other analyses, repeated sampling of the impedance signal costs almost nothing and herein lies it major

attraction. By good fortune (though early impedance workers may not have realized it) the impedance signal from ordinary levels of microbial contamination is too small to be instantly detected. Manufacturers of impedance-based instruments have been forced to design incubation steps into their analytical schemes and thus give the organisms a chance to prove their mettle.

There are two problems. Perhaps of least significance, the broths needed for easy impedance measurement differ chemically and physically from foods leading to some doubt as to how well the behavior of florae in impedance broths describes their behavior in food.

Of greater significance in 1986, it must be admitted that for the microbiologist wishing to know the count, the impedance detection time (IDT) appears to be a marginally useful substitute. One need only glance at impedance/CFU scattergrams to understand: the general trend of a line is always visible but the points often run amuck. This is indeed a knotty problem for manufacturers of impedance equipment. Survival dictates the sale of instruments that count, or at least that reduce the microbiologist's need to count. Some very valid proposals from impedance merchants — schemes for screening out the very good samples and the very bad, leaving only the questionable ones for counting — should be catnip to anyone charmed by displays of human ingenuity.

For the moment this is how it must be. Counts are the way we measure food quality and they must figure in the performance of microbiological automata, or else the instruments will not sell. But it is encouraging that impedance people are quietly working towards popularizing a more rational concept of their products; the ability of metabolic measurements to predict shelf-lives.[17-19] While it may not be the best parameter to use for shelf-life prediction, it must be seen that the impedance datum is as much a function of microbial virility as of microbial number. This, of course is why it does not convincingly predict the microbial count; it contains more information about the flora than the count can give. Thus, it is more correct to say that the plate count does not adequately describe the IDT. In time, and as impedance instruments become more common, this viewpoint may become more widespread: at the moment the impedance people must sell counts to stay in business. But selling such nebulous images as "IDT" or "percentages of samples outside the grey zones" in the face of so tangible a concept as "numbers of CFU" is an unenviable task.

Yet impedance, and the salutory effect its proponents are having on microbiological thinking, must be seen as only one step in a rationalization of the predictive side of analytical food microbiology. Of necessity, the impedance growth media do not accurately represent the microbial environment within the food. Nor do the various hydrolyses and dissociations responsible for impedance change relate directly to the factors responsible for the consumer's sensory perceptions or susceptibilities.

That is to say, many of the hazards, and certainly all of the spoilage parameters to which the consumer responds are simply physical, chemical, biochemical, or immunological manifestations of microbial growth and activity. And of these, the ionic and other changes detectable by impedance meters are a rather small subset. Thus, although an IDT may tell us more about microbial puissance than does a plate count, it cannot necessarily be counted upon to tell us that the consumer will succumb to toxin in 2 days or reject the food in 5 days on account of its acetoin taint.

There is another approach, an extension of the metabolism-based technique, that I believe offers the ultimate in terms of meaningfulness for microbiological data.[4] It demands automation and computerization. It has the potential to tell us, for example, that release of a product for sale would result in 17% of odor complaints after 10 days in the refrigerator, or 75% incidence of toxicoses after a night on the counter, and so on (data the microbiologist could really stand behind.)

Unfortunately, this approach demands the compilation of so much background data (some of it not easy to obtain) from which the analytical instrument would arrive at its prognosis,

that it will probably never become a universal analytical technique. Yet I see attempts here and there to develop predictive models that go some way towards this end.[20-27] Most of the development is being carried out confidentially in large food manufacturing establishments. In fact, it is directed more towards my final discussion subject, control of manufacturing processes to eliminate the need for microbiological testing.

For any food the main steps in the predictive approach are as follows:

1. Database Experiments

1. Decide on a few important microbial parameters (manifestations) responsible for quality loss or hazard (for example, short chain fatty acids, hydrogen sulfide, color change, fluid loss, shear strength).
2. Obtain amplitude/response graphs for these parameters. For example, by chemically altering to particular levels, the short chain fatty acid content of specimens used in organoleptic evaluation panels.
3. Lay down, through consultations on manufacturing costs for various standards of hygienic processing, effects of complaints on brand image and profitability, etc., a maximum tolerable amplitude for each parameter in the food. When a parameter has grown to this amplitude the food is at the threshold of unwholesomeness with respect to that parameter. We must now assume the existence of the automated analytical instrument for the next step.
4. Prepare a body of data from specimens of the food, from which the mathematical equations of the envelopes describing the growth of these parameters (with confidence limits) under different conditions can be derived. For example, the envelope describing hydrogen sulfide and ammonia evolution during the deterioration of ground beef, as a function of time and temperature through the range 0 to 30°C, might be derived. Or, as a corollary, envelopes might describe the shelf-life (time to reach a threshold of unacceptability) of ground beef as a function of temperature, for each parameter.

2. Predictive Modeling Analyses

With the database complete for the time being, many types of predictive samplings are possible. I use here an example of shelf-life predictions. Routine analyses require a plurality of well-mixed subsamples of the specimen to be added to the instrument. The samples are incubated under a range of conditions differing from, but converging on, the normal storage condition of the food. For example, if the food is normally stored at 5°C, the instrument may be set to incubate subsamples at 5, 10, 15, 20, 25, and 30°C. It will then proceed to make analyses for the chosen parameters at frequent intervals.

The process is more complex than this, but the theory is straightforward. The time required for a parameter to develop to the threshold level is a datum combining information on all six of the unknowns discussed in Section IV.A. Thus, it is a function of the original number of cells, their ability to multiply in the food, their vigor at producing the chosen parameter, and the effects of synergism and antagonism. It does not relate merely to the instant at which the food was sampled but to the whole microbial growth period. And, in particular, is also related to the human response function.

If the analysis was carried out only at 5°C it would yield a very accurate, though not very timely prediction of the shelf-life of the product. However, at the higher incubation temperatures things happen faster. In the ideal case for the chosen specimen, an analyzed parameter develops most rapidly at 30°C and increasingly slowly at lower temperatures. As soon as the parameter reaches its threshold at 30°C the instrument makes its first prediction of shelf-life. (In fact, it can start doing so long before the first threshold is reached).

This first (earliest) prediction is the least accurate or precise. As samples closer to 5°C begin to yield data the instrument increasingly firms up its predictions. The microbiologist