Biotechnology: Approaching a Critical Mass

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It was about 1965 when the flavor industry came to an important crossroad, a time for a decision concerning its financial allocations and emphasis in research for biotechnology.

At that time the industry observed some statistical consumer information indicating a possible trend toward "natural flavors," some signs, as it were, that there might be a genuine need for a resource commitment but no concrete evidence that guaranteed where the consumer was heading. Some in the industry believed that the cry for "naturals" was just some kind of mystery market that would pass, a temporary chemophobia. For others, the vision urged them to move forward with research. Nineteen sixty-five then was the year when only a few companies committed resources and decided to use biotechnology to produce flavors.

The biotechnology front for the flavor industry remained relatively quiet until about the mid-eighties when the "natural" rush really started. By 1984 the cry for "natural" was not a statistical trend anymore, it became a fact.

Today we conclude it is here to stay! Food industry's requests in 1985 for new natural flavors tripled those of the 1965 while the demand for artificial flavor decreased dramatically (see figure 1). Fifteen years have passed since some commercial "natural" building block such as amyl acetate and ethyl acetate was produced by biotechnology; therefore it is appropriate to review the progress today and try to envision the future.

In the early stages of planning to create natural flavors, Nature's own way of producing its flavors was considered first. The already available natural building blocks, essential oils, fruits and spices, were in common use. Their costs and functional limitations were fully understood. What was really needed was to study Nature's way of producing its metabolites. Since flavor chemicals are produced as metabolites in plant and animal cells or through microbial actions, it was quite evident that fermentation was the best available route (see figure 2). Literature, patents

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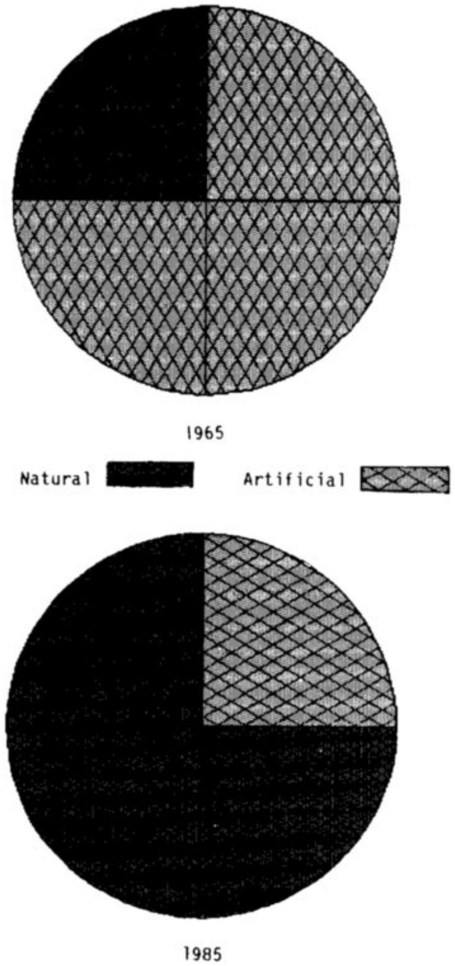

Figure 1. Request for Flavors from the Food Industry (Givaudan Statistics)

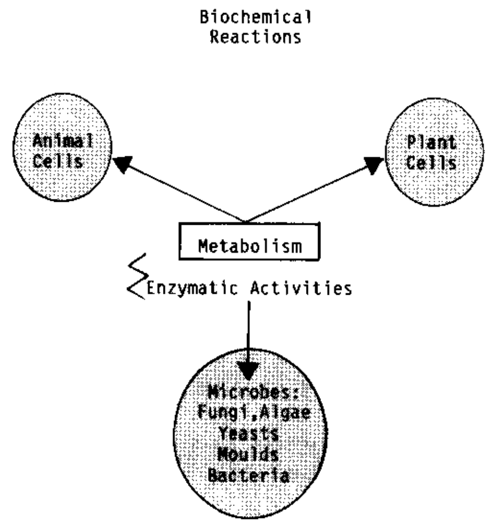

Figure 2. Enzymatic and Microbial Reactions

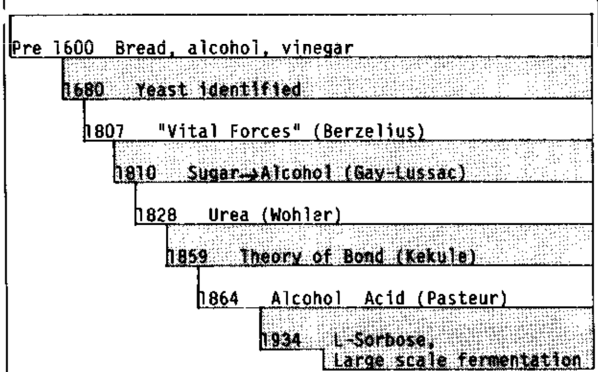

Figure 3. Fermentation History

and technical reports dealing with enzymatic and microbial reactions were accumulating. The long history of fermentation was encouraging, especially when it was shown about 1934 that L-sorbose could be prepared by large scale fermentation (figure 3).[1,2]

As far back as 1959, the United States Department of Agriculture reported fourteen types of fermentation reactions.[2] Such reports were strong motivation for the flavor industry to start its basic research for natural ingredients.

The progress from 1965 to 1986 was rather slow; less than one hundred chemicals composed of acids, esters, few lactones and aldehydes were available to flavor chemists. (This is easily quantified by reviewing current brochures and publications of various suppliers.) Certainly these few chemicals are not impressive when we consider the thousands of metabolites produced by Nature.

Demand for New Naturals

We have the promoters who are the suppliers of enzymes on one hand, and the contract researchers on the other. Both are claiming that microorganisms can do it all. The flavor industry is now awakening and facing the reality that the slow progress in the further development of fermentation products is not keeping up with the increasing demand for new naturals.

The slow progress in producing flavor components can be attributed to varied causes. In the first place, interest for natural flavors, until recently, was mainly a North American trend. Moreover, some European legislators did not agree with the FDA definition for "naturals." This prevented serious research from starting up in other parts of the world and minimized the positive impact which open interchange of ideas and healthy global competition foster. However, this situation is now changing, both in European consumer demand for natural flavors as well as in legislation foreseen by the new Codex Alimentarius definition of "naturals".[3]

The low sales potential of flavor components is a second reason for giant chemical companies to seriously consider research in this area unattractive. Very few building blocks have the magnitude value of $100 million dollars per year represented by vanilla, mints or citrus. In fact, the majority of components have an estimated dollar value of less than one million dollars per year.

The impressive number of about 2000 enzymes identified by scientists brings a third illusion to the biotechnological dream.[4] Monographs citing hundreds of formula schemes leave the application scientists with more questions than answers.[5] In most cases the enzymes are not commercially available, and the metabolite yield is too low to make the transformation practical. Moreover, in many cases, the necessary natural substrate for a particular fermentation is not available.

Fourth, screening and optimizing several conditions of fermentation reactions, such as pH, nutrient balance, co-enzymes, co-factors, are tedious processes. Although partly scientific, it is to a large extent mostly trial and error which extends the time of product realization to several months.

The next important factor is that some secondary metabolites could denature enzymes or act as microbial inhibitors, thus preventing high yields required in fermentation reactions. (Vanillin is a typical example of this difficulty.)

Finally, the available alcohols and acids produced by fermentation that are currently available represent the simpler part of the primary metabolism. Secondary metabolites constitute the more challenging task.

Plant Cell Cultures

Such difficulties encountered in fermentation reactions warrant the consideration of other techniques. A new approach is needed, not to replace fermentation, but rather to support it. Several scientists thought plant cell cultures could be the other support route. It is a logical solution to attempt to induce the intact cell to do the job for us.

Plant tissue culture (PTC) is neither farfetched nor totally novel. The concept of "cell theory" has been brewing since 1839 (figure 4).[6] The National Research Council of Canada was among the pioneers in the investigation of PTC for flavor production. However, we have to ensure that the recent enthusiasm surrounding this technology does not create another industrial illusion. Several considerations will help realize the long and tedious road ahead of us before we reach a breakthrough.

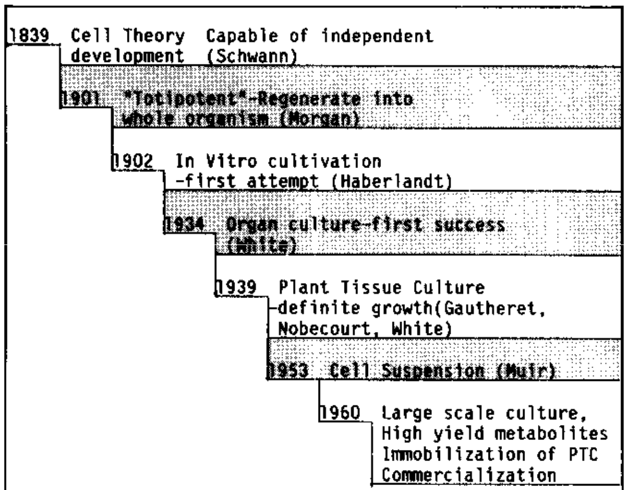

Figure 4. History of Plant Tissue Culture

We know, first, that plant tissue culture technique is nothing more than a very complex fermentation process. Cell growth and reproduction is much slower than microbial fermentation. Moreover, secondary metabolites production is generally not growth associated; therefore, optimizing culture conditions and media is as difficult as, if not more complex than, fermentation. Besides the strict requirements for aseptic conditions, several parameters of culture media must be optimized mostly by trial and error. These parameters include nitrogen, phosphate Ca^{++}/Mg^{++}, other salts, O_2/CO_2, carbon source, hormones, light, pH, temperature.[7]

Second, cells exhibit much shorter terms of survival in the process of continuous cultivation than microbes do.

The third consideration, the uncertainty of legislative status concerning products produced by PTC, further clouds the issue. FDA's L. Robert Lake told a recent Food and Drug Law Institute/FDA educational conference in Washington that there are no clear FDA guidelines on food additives' GRAS status for a substance made through biotechnology. He stated however, that, if the substance is "substantially identical" to the GRAS status of its conventional counterpart, it stands a good chance of not losing that status.[8]

On the other hand, the tedious road has some good signposts. Some factors which were cause for skepticism on the feasibility of PTC until 1983 are breaking down. The announcement of the Mitsui Petrochemical Industries, for example, of their 750 L. reactor to produce Shikonin by culturing Lithospermum erythrorhizon cells, made many believers and created a new interest.[9]

During the last few years, many joint agreements between food or flavor manufacturers and universities or research institutes were signed.[10]

Although we should keep an objective view toward these encouraging developments, several other positive signs indicate the way to widespread use of PTC biotechnology. It is still difficult, however, to predict results—a breakthrough might be accelerated by serendipity. We see, however, a possible critical mass for success.

Elements for Success

Literature reports provide indications that some metabolites accumulated from PTC optimized cultures are in yields close to or higher than the parent plant, by factors ranging from 1 to 173 times.[11] Increasing reports of successful continuous high yield process production makes PTC more feasible for flavor production.

Next, cell immobilization became a reality as described by Brodelius reporting successful immobilization in alginate, Agarose, carrageenan, polyurethane foam and other materials.[11]

Furthermore, recent technological advances are shifting the economic feasibility of phytoproduction towards a more realistic dollar potential. According to Goldstein et al., a five year payout on invested capital can be attained at a selling price of $43/Kg for a metabolite, corresponding to a one million Kg per year metabolite production and $17/Kg manufacturing cost.[12]

In addition to these elements, recent strong interest in natural flavors in many European countries will increase global research and a more healthy level of competition. Moreover the interest in the plant kingdom as a source of medicine will certainly direct some research in this area.[7]

We must also view the possible application of the high technology of gene splicing to cells providing them with more productive features of microbes or enzymes. It is an application that looks very promising.

Conclusion and Future Outlook

The salient elements for bringing about a critical mass in the development of biotechnology for the flavor industry are evident. However, I believe that the major factor hindering rapid progress is the lack of basic scientific knowledge on microbial and cell metabolisms.

Our governments as well as the industry should encourage basic scientific research, both in universities and nonprofit research centers. The lack of funds in these institutions is pushing scientists' work toward applied science more than basic science. The old style of research of the 1940s, with the concept of science for the sake of science is gradually being "phased out." Very little work on fundamental laws issued from today's ivory towers. We are missing the Avogadro's type of theory, the Kekule Hypothesis and the basic research carried out by Pasteur. Basic research is what made our industrial development forge ahead and initiated the breakthroughs in the classical chemistry of yesteryear.

In the field of biotechnology, the basic understanding of the complex factory of the living bacteria and cell is of utmost need. Such an undertaking will never be on any agenda of an industrial research institution and will not be seen in joint projects paid for by the industry. Unless we replace the entrepreneurial with the basic type of research in our universities and research councils, progress will be at a standstill.

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