Are Microbes Optimal Strategists?

Several published experiments in the literature have provided main guidelines for a quantitative modeling approach called "cybernetic modeling." Cybernetic models have correlated bacterial growth with mixed substrate systems with remarkable accuracy.

Doraiswami Ramkrishna, Dhinakar S. Kompala, and George T. Tsao
School of Chemical Engineering and Laboratory of Renewable Resources Engineering,
Purdue University, West Lafayette, IN 47907

Introduction

In an earlier paper [1] on the experimental evaluation of cybernetic models for bacterial growth on multiple substrates, we presented certain basic postulates based on all existing experimental literature known to us that all models for bacterial growth in a multisubstrate environment must satisfy. At the suggestion of one of the reviewers of that paper, we are presenting an analysis of the experimental literature that led us to the elucidation of the postulates mentioned above. It is our belief that this analysis will provide a greater understanding of the optimal nature of cellular regulatory processes controlling the bacterial growth on multiple substrates.

Bacterial growth is a highly regulated process involving thousands of biochemical reactions. Some of these regulatory actions become strikingly apparent during the growth of bacteria on media containing more than one functionally equivalent (i.e., serving the same physiological function, such as carbon source, energy source, nitrogen source, etc.) substrates. The regulation of multiple substrate utilization was detected as early as the turn of this century, when Dienesert [2] found that glucose had an inhibitory effect on the galactose fermentation by yeast. Similar interactions between carbohydrates were reported by some other early investigators [3], [4]. A systematic and extensive study of bacterial growth on multiple substrates was conducted by Monod [5], who discovered the phenomenon of "diauxie" and established its close relationship to enzymatic adaptation. Since that time, numerous researchers have observed the sequential utilizations of multiple substrates by a variety of microorganisms. A close scrutiny of many of these reports shows several basic characteristics apparent in all the observations of bacterial growth on multiple substrates and are listed below.

Basic Characteristics of Bacterial Growth on Multiple Substrates

1. Given multiple substrates, bacteria prefer to utilize the substrate on which they can grow the fastest, commonly resulting in a sequential utilization of the substrates in batch cultures.

2. Sequential utilizations turn to simultaneous utilizations, even in batch cultures, when the carbon and energy sources are available in such high concentrations that some other nutrient source becomes rate-limiting.

3. Even during the simultaneous utilization of multiple substrates, the total growth rate is never greater than the highest of the growth rates on each of the substrates.

4. While growing on a slower* substrate, if a faster substrate is added to the culture, the growth on the slower substrate is quickly regulated through catabolite inhibition and repression of the enzymes involved.

5. In continuous bacterial cultures, multiple substrates are consumed simultaneously at low dilution rates, and the faster growth-supporting substrate is consumed preferentially at high dilution rates.

In this review paper, we present some of the key experimental observations from the literature that provide a clear justification for our formulation of the above characteristics.

Characteristic 1

Following the pioneering work of Monod [5] on the growth of bacteria on multiple substrates, it has been recognized that the growth behavior ranges from simultaneous utilization of all substrates to sequential utilization of the substrates in some order of preference. Figure 1 shows the growth of Escherichia coli in the presence of different carbohydrate pairs serving as the only carbon sources in a synthetic medium. Monod termed the sequential utilization of two substrates in two exponential growth cycles separated by an intermediate lag period as the "diauxie" and the lag period as the "diauxic lag." It has been assumed that the utilization of the two substrates is simultaneous when a diauxic lag phase is not apparent in the growth curve. However, there have since

* The adjectives slower and faster preceding substrate obviously refer to growth on the substrate rather than to the substrate itself.

Dhinakar S. Kompala is presently at Department of Chemical Engineering, University of Colorado, Boulder, CO 80309-0424
FIGURE 1. Growth of E. coli on different parts of carbohydrates. From Monod [5].

![Growth curves for different carbohydrates](image)

been many well-documented observations of sequential utilizations of two substrates without any apparent diauxic lag phases in the growth curve. For example, Standing, et al. [6] measured the substrate concentrations during the growth of E. coli on glucose and galactose and found that glucose was completely utilized before galactose was consumed, even though the growth curve did not exhibit a diauxic lag phase. Similarly, Clark and Holms [7] found that E. coli grew on glucose and fructose sequentially without any diauxic lag. These observations are indicative of more widespread occurrences of sequential utilizations than may be obvious from the growth curve alone.

In all the early observations of sequential utilizations, the commonly used enteric bacteria consumed glucose first, and the other substrates subsequently. This has resulted in an interpretation of these phenomena as the “glucose effect.” However, there have since been several observations of sequential utilization of two substrates by other organisms with glucose being consumed second. Some examples of this type are Pseudomonas aeruginosa growing on lactate and glucose [8], Arthrobacter cristallopoiesis growing on succinate and glucose [9], Propionibacterium shermanii growing on lactate and glucose [10], and Thioacillus A2 growing on succinate and glucose [11]. The mislabeling of these observations of preference for substrates other than glucose as the “reverse diauxie” [12] unfortunately perpetuates the earlier misinterpretation of diauxic growth as a glucose effect. It becomes quite clear from other experimental studies that the diauxic growth phenomenon is not linked at all to the bacterial consumption of glucose. For instance, Pseudomonas oxalaticus grows diauxically on a mixture of two organic acids, acetate and oxalate, consuming acetate during the first growth phase and utilizing oxalate only after an intermediate lag phase following the virtual exhaustion of acetate [13].

In all these different studies on sequential utilization of multiple substrates, there is one strong correlation between the order of preference and the specific growth rate during each growth phase. In all the cases the preferred substrate is found to be the one which the bacteria grow faster. This correlation is most dramatically illustrated by the results of Lee, et al [10]. They found that the maximum specific growth rate (μmax) of Propionibacterium shermanii on glucose as the sole carbon substrate is 0.141 hr⁻¹ and the μmax on lactate as the sole carbon substrate is 0.142 hr⁻¹. With such a small difference in the growth rate of the two single substrates, the bacteria utilized lactate preferentially, when presented with a mixture of lactate and glucose. In another well-characterized study, Wood and Kelly [11] found that Thiobacillus A2 prefers the organic acid fumarate (μ = 0.514 hr⁻¹), succinate (μ = 0.460 hr⁻¹), or acetate (μ = 0.374 hr⁻¹) over glucose (μ = 0.126 hr⁻¹) in a diauxic growth pattern. Similar examples of bacterial preference for faster growth supporting substrates are quite numerous, and we are not familiar with any experimental observations to the contrary. Such a strong correlation between the growth rates and the order of preference between multiple substrate is indicative of an invariant strategy of microbial regulatory processes, such as repression and inhibition, to optimize the cellular growth rate.

For the more common observations of the diauxic growth phenomenon with the enteric bacteria that usually consume glucose faster than any other substrate, it is not surprising that glucose is often the preferred substrate. However, the diauxic growth phenomenon appears to be truly associated with the bacterial preference for consuming the fastest growth supporting substrate, which need not generally be glucose. Thus, the labeling of the preference for citrate over glucose by Pseudomonas aeruginosa, which grows on citrate faster than on glucose, as “reverse diauxie” is inappropriate [14] and should be discouraged. In observations of multiple exponential growth phases without any intermediate lag phases [15], [16], the specific growth rate of the batch culture decreases monotonically with each successive growth phase. In many industrial fermentations on media enriched with nutrients, vitamins, and other growth enhancers, a monotonic reduction in growth rate is commonly observed. Although the successive growth phases merge together as the vitamins and other growth enhancers are gradually exhausted, which again leads to the conclusion that the microorganisms consume preferentially those nutrients supporting the faster growth rate.

Characteristics 2

This characteristic is the latest addition to the four basic postulates we put forward in our earlier paper, which was mainly concerned with an experimental evaluation of cybernetic models [1]. It was clearly established therein that Klebsiella oxytoca grew diauxically on glucose and xylose, when the total sugar concentration was not allowed to exceed 10 g/l, such that the carbon and energy source was the only limiting nutrient. However, Jansen [16] found that the same organism consumed the two sugars simultaneously when their concentrations were increased to about 100 g/l each, and the salts and trace minerals concentrations were left unchanged at low levels. Thus, sequential utilization of glucose and xylose by K. oxytoca becomes transformed to a simultaneous utilizati-
tion of both sugars when the carbon and energy source is no longer the limiting nutrient.

Similar transformations between sequential and simultaneous utilizations of glucose and lactose were observed during the growth of the yeast Kluyveromyces marxianus [17]. While much of the experimental literature analyzed here pertains to the growth of bacterial cultures on multiple substrates, each of the characteristics presented is equally representative of the growth of yeast cultures as well. As thoroughly documented experimental observations concerning Characteristic 2 are scarce in bacterial literature at large, it is useful to consider the results from yeast growth studies. Wang, et al. [17] found that K. marxianus consumed the two sugars, glucose and lactose, in different patterns when cheese whey powder or yeast extract (either of which supplies all the other required nutrients) were provided at different levels. At higher levels of these additives the sugars were consumed sequentially, and at lower levels of the additives the sugars were consumed simultaneously. These observations indicate a general trend that sequential utilization of multiple carbon substrates is predominant when the carbon source is the rate-limiting nutrient, and that the utilization becomes simultaneous when some other nutrient becomes limiting.

From a genetic perspective that bacterial growth on multiple substrates is a phenomenon of instantaneous optimization of the specific growth rate of the culture, the above observations of transformation from sequential to simultaneous utilization of multiple substrates when some other nutrient becomes rate limiting may be explained as follows. When only the carbon and energy substrates are rate limiting, it follows that all the other required nutrients are present in such high concentrations so as not to limit the rate of the growth processes. The growth rate of the culture in such an environment is controlled by the rate of production of the precursors, required for macromolecular synthesis, through the catabolism of the carbon and energy substrates. Multiple carbon substrates are available in the environment, from which the precursors are produced at different rates through the different catabolic processes, then it becomes crucial for the bacteria to prefer only the substrate that can be catabolized faster to maximize the rate of the overall growth process. On the other hand, if the carbon substrates are present in excess and some other nutrient source, say an ammonium or phosphate salt, is in short supply such that overall growth rate is limited by its availability rather than the availability of carbon precursors for the macromolecular synthesis, then it becomes unnecessary for the bacterial regulatory processes to choose between the multiple carbon substrates as each may be able to supply the carbon precursors at the required rates. In such a situation, an optimal regulatory network would allow a simultaneous utilization of the multiple carbon substrates.

**Characteristic 3**

This characteristic can be derived most clearly from continuous culture data, as simultaneous utilization of multiple substrates is more predominant in chemostat fermentations than in batch cultures. The maximum specific growth rate of pure cultures during batch growth on a single substrate sets an upper limit on the maximum dilution rate at which a continuous bioreactor can be operated with the same single substrate being fed continuously, before the washout of the culture sets in. When multiple substrates are fed to the continuous bioreactor the maximum dilution rate remains the same as the maximum dilution rate possible when only the fastest growth supporting substrate was fed to the chemostat [18], [19]. This observation suggests that the maximum specific growth rate possible on multiple substrates is no greater than the maximum specific growth rate on the single substrate supporting the fastest growth.

In batch cultures, simultaneous utilization of multiple substrates is very rarely observed, except as described in the discussion of Characteristic 2. In both those cases, the specific growth rate during the simultaneous utilization is no greater than the maximum specific growth rate possible on the fastest single substrate. In all the well-documented experimental observations of sequential utilization of two or more substrates, the faster substrate in the mixture known to be present in large excess is first utilized, while the slower substrate would necessarily be completely consumed only when the faster substrate is still present in adequate amounts. However, shifting from the faster substrate to the slower substrate would cause the overall specific growth rate of the cell to decrease, and it is not surprising that bacteria, which have been shown to maximize their growth rate whenever possible, do not initiate their catabolic assimilation of the slower substrate while the faster substrate is still present in adequate measure in the environment. On the other hand, when a preferred substrate is added to the culture during an exponential growth on the less preferred substrates, then the growth rate responds immediately and attains the rate corresponding to the growth on the faster substrate alone [1].

Here again, the overall specific growth rate of the culture would have been slower if the cellular reaction network continued to catabolize the less preferred substrate than if it switched its catabolic enzyme pool completely to the utilization of the faster substrate. Any intermediate switch, where catabolism of both the substrates is carried out, would result in the overall specific growth rate of the culture being weighted average of the growth rates on each substrate. The growth rate on multiple substrates is thus never greater than the maximum growth rate on the fastest single substrate present.

There are only two experimental results in the literature known to us that may appear to suggest an additive growth on multiple substrates. However, on closer inspection, these data can also be resolved to be noncontradictory to Characteristic 3. Stumm-Zollinger [20] observed that a heterogeneous bacterial population grew faster (additively) on a mixture of glucose and benzoate than on each of the substrates alone. This observation is really not a contradiction, since the culture contained multiple species. Such an additive growth would result for a population containing just two species, if one species prefers one substrate and the other species prefers the other substrate. Wood and Kelly [11] reported that Thiobacillus A2 exhibits additive growth (p = 0.22 hr⁻¹) on a mixture of glucose (µ = 0.126 hr⁻¹) and galac-
glucose (μ = 0.135-0.207 hr⁻¹ for gal. conc. of 10-30 mM). They also reported that the growth rate on glucose alone increased to 0.4 hr⁻¹ after 3 or 4 sequential subcultures, equivalent to 12-16 population doublings. This faster strain was shown not to exhibit any enhancement of growth rate on addition of galactose. Hence, their report of “additive” growth is suspected to be the result of an uncharacterized strain improvement with the concomitant faster growth on glucose alone.

Characteristic 3 is believed to be well founded for pure cultures of bacteria, and forms the justification for a key assumption of the cybernetic perspective [21], [22], [33] [24]. Namely, that certain critical resources are used up during the growth on any substrate in the production of catabolic enzymes, and for multiple substrate growth these resources must be optimally distributed for the synthesis of the catabolic enzymes for growth on each of the substrates. The optimal goal of this distribution is then assumed to be that of maximization of the instantaneous growth rate of culture, which results in a preference for the faster growth-supporting substrate, as shown in the discussion of Characteristic 1.

**Characteristic 4**

This characteristic addresses the cellular regulatory processes, which are viewed in the cybernetic framework as the strategies through which the growth rate optimization is achieved. Soon after his pioneering studies on bacterial growth on multiple sugars, Monod [25] addressed the mechanistic causes for sequential utilization and identified the phenomenon of “enzymatic adaptation” as the primary factor. Working with several *E. coli* mutants constituted for lactose consumption, his studies led to the discovery of the lactose operon, which is repressed by the presence of glucose and induced by lactose in the absence of glucose. It is now recognized that the repression is mediated not directly by glucose but through its catabolites, hence this phenomenon was named the “catabolite repression” [26] rather than an earlier misnomer “glucose effect.” To emphasize the correlation between the repression of enzyme synthesis and the growth rate on single substrates, it is worthwhile to point out that any substrate that supports a faster growth rate than that on lactose causes a repression of the lactose operon in *E. coli*. Similarly, for *P. aeruginosa*, which grows faster on citrate than on glucose, Ng and Dawes [27] found that the enzyme synthesis is repressed whenever citrate is present in the medium and as the citrate concentration is reduced, the enzymes for glucose metabolism are gradually induced. Thus the repression of the enzymes necessary for growth on a slower growth-supporting substrate, when faster substrate is present, appears to be an invariant strategy of the cellular regulatory network to optimize its growth rate.

In addition to the control of enzyme synthesis through the processes of induction and catabolite repression, the cellular regulatory processes include mechanisms for quickly reducing the catalytic activity of the enzymes already present for the metabolism of the slower substrate, when a faster substrate is added to the culture. McGinnis and Paigen [28] clearly demonstrated the phenomenon of catabolite inhibition of the enzymes involved in utilization of lactose and galactose, when glucose was added to the *E. coli* cultures. This inhibition is distinct from catabolite repression because the rate of slower substrate utilization is reduced to a level below that observed prior to the addition of glucose. Immediately after the removal of glucose from the culture, the rate of lactose or galactose utilization recovered to the level prior to glucose addition, showing that certain key enzymes involved in the slower carbohydrate utilization are reversibly inactivated. The cellular regulatory processes of catabolite repression and inhibition controlling the synthesis of new enzymes, and the activity of the already existing enzymes for the catabolism of the slower substrate thus, constitute the invariant strategies for the cellular reaction network to maximize its overall specific growth rate instantaneously.

**Characteristic 5**

In contrast to the sequential utilization of many carbohydrate pairs (see Figure 1) by *E. coli* in batch cultures, the substrates are consumed simultaneously in chemostat cultures at low dilution rates. As the dilution rate was increased, Mateles, et al. [18] found that the culture gradually shifted to a preferential utilization of glucose, which is the fastest growth supporting substrate for *E. coli*. Harte and Webb [29] found that glucose and maltose, which were sequentially utilized in batch cultures by *Klebsiella aerogenes*, were consumed simultaneously in continuous cultures at low dilution rates, and as the dilution rate was increased, the time lag before the utilization, or maltose since its introduction increased, until at high dilution rates maltose was not consumed at all. Recently Harder and Dijkhuizen [30] studied the growth of *Pseudomonas oxalaticus* on a mixture of acetate and oxalate. In batch cultures, “high” growth was observed when the preferred substrate was acetate being first. In continuous cultures at dilution rates below 0.15 hr⁻¹, the organism utilized both the organic acids simultaneously and completely. At dilution rates above 0.15 hr⁻¹, an increasing amount of oxalate remained unused. In contrast, no residual acetate was detected in the culture supernatant up to a dilution rate of 0.30 hr⁻¹. The same type of behavior was observed in chemostat studies of yeast cultures as well [18], [19]. All of these observations clearly form the basis for Characteristic 5.

Caution has been sounded against this basic characteristic, due to one apparent exception to the rule. Standing, et al. [6] have claimed that *E. coli* consumed both glucose and xylose completely: “at both high and low dilution rates.” However, the “high” dilution rate they have used is only 0.16 or 0.166 hr⁻¹, which is less than 20% of the μmax for xylose. All the preferred utilizations by *E. coli* have been observed at dilution rates higher than 50%, more typically 75%, of μmax of the less preferred substrate, hence the data of Standing, et al. [6] do not conflict with the observations of preferred utilization at high dilution rates mentioned for Characteristic 5.

Silver and Mateles [31] demonstrated that catabolite repression and inhibition of enzymes for lactose metabolism play crucial roles in the preferential utilization of the faster growth-supporting substrate, glucose, at high dilution rates. Catabolite repression of enzymes for glucose metabolism by the faster growth supporting citrate was demonstrated by Ng and Dawes [27] in chemostat cultures of *P. aeruginosa*. Similarly, Clarke, et al. [32] observed catabolite repression of aliphatic amidase, an enzyme is necessary for the assimilation of acetamide, in continuous cultures of *P. aeruginosa* on succinate and acetamide. Thus, the same regulatory processes that cause sequential utilization and diauxic growth in batch cul-
tures are manifest in continuous cultures as simultaneous utilization at low dilution rates and preferential utilization at high dilution rates.

In terms of the optimizing nature of microbial growth, this general trend in continuous culture may be interpreted as follows. In chemostat cultures, the specific growth rate of cells is restricted by the low substrate concentrations to be equal to the dilution rate at which the chemostat is being operated, hence the cellular regulatory network cannot be viewed as optimizing the specific growth rate of the culture at each instant. However, the regulatory network is still functioning in the chemostat cultures to ensure an optimal distribution of the critical resources for the synthesis of the catabolic enzymes necessary for growth on each substrate. At low dilution rates, either substrate alone can provide the low growth rate as the rate of catabolic breakdown if either substrate is not rate limiting; thus the cellular regulatory processes are not invoked to select only one of the catabolic processes. The cell concentration at low dilution rates is indeed an addition of the growth yields on each of the substrates [29].

As the dilution rate is increased however, the rate of the catabolism of the slower substrate to supply the precursors for the macromolecular synthesis is more limiting than the rate of the catabolism of the faster substrate. It therefore becomes crucial for the optimal regulatory processes to gradually increase the synthesis of the enzymes for the faster substrate and to reduce or repress the synthesis of the enzymes for the slower substrate.

Cybernetic Models

The postulates discussed above were built into a mathematical framework [1], which broadly consisted of the following features. Growth on each substrate was described by Monod-saturation kinetics modified by two multiplicative factors. One represented a "key enzyme" level (say e) for metabolizing substrate (S) and the other a "cybernetic" variable (say v), which controlled the activity of that enzyme. The enzyme synthesis rate was also described by Monod-saturation kinetics modified by another cybernetic variable (v), which controlled the synthesis rate of that enzyme. The cybernetic variables were calculated using microeconomic principles. Thus, Herrnstein's matching law [32], which states that the fractional allocation of a common resource among several alternatives (satisfying the law of marginal returns) for a particular alternative is equal to the fractional return from that alternative, was used for calculating the cybernetic variables (v). A slightly different allocation policy was used for the cybernetic variables (v) necessitated by Characteristic 3. Small values of v represented repression of that enzyme while values close to unity represented active induction. The cybernetic variables (v) represented allosteric control of enzyme activity, small values representing inhibition and values close to unity, denoting activation. Metabolic regulatory processes were thus accounted for by the cybernetic variables whose values were computed from simple economic principles. The framework allowed for identification of the model parameters from growth on single substrates only. Growth on mixed substrates was described without further adjustment of model parameters by using cybernetic variables; for details refer to Kompala et al [1]. Figure 2 shows a triauxic growth curve predictions by the model alongside experimental measurements on Klebsiella oxytoca. Turner [34], and Turner, et al [35, 36] have subsequently incorporated maintenance effects that have substantially enhanced the predictive capabilities of the model [1].

FIGURE 2. Growth of K. oxytoca on a mixture of glucose, xylose and lactose. A simulation of cybernetic model versus experimental data. From Kompala et al [1].

FIGURE 3. Response of a glucose-limited fed-batch culture to the addition of 0.4 g fructose at t = 4.1 hr. From Turner [34].
rates, growth on the less preferred substrate occurs after a lag period not predicted by the model. This indicates the direction of some future changes in the cybernetic models, which have so far provided for resource allocation without a resource balance. When resources become rate limiting, lags such as those observed by Turner, et al [35, 36] are likely to be manifested.

Discussion

In the formulation of the various characteristics presented herein we have clearly been guided by certain simple experimental situations. From a pedagogical point of view, it would be of interest to identify the least number of independent characteristics in terms of which the behavior in all experimental situations can be described. At this evolutionary stage it is neither possible nor desirable to identify such a minimal set of characteristics. There are, for example, a number of issues we have not discussed here that have already been addressed elsewhere. We have not elaborated on the issue of maintenance requirements of the microorganisms that Turner, et al [35, 36] have recently considered in detail, using cybernetic models with considerable success. These models predict maintenance effects not treated by the models of Kompa, et al.

Another issue concerns that of constitutive versus inductive enzymes, which has been considered by Turner and Rankrnskha [37]. This issue is also an important one, and the models of Kompa, et al [1] must incorporate the changes made by Turner and Rankrnskha [37] in order that certain continuous culture transients be described properly.

Fredrickson has pointed out [38] that the mixed substrate systems that have been addressed by us need to be qualified as being constrained essentially to "substitutable" substrates and that a generalization envisaging the description of growth in complex media (such as those in industrial fermentations) must account for both substitutable and "complementary" substrates. We believe that the discussion of Characteristic 2 has some contributions in this regard, but undoubtedly some further refinement is possible.

Finally, we wish to point out that the postulates here must be viewed as no more than a preliminary effort which, based on the success it has had, looks promising. Qualitatively, ideas of cells being efficient systems, have been proposed before [39]. The cybernetic models have shown that such notions could have quantitative significance. It would seem unessential, however, to be prewarned of the fallibility of an omnipotent set of postulates concerning the behavior of living systems.

Literature Cited

34. Turner, B.C., Ph.D. Dissertation, Purdue University, 1984.

Doraiswam Ramkrnskha is a professor of chemical engineering at Purdue University. He received his B. Eng. degree in chemistry (1960) from the Bombay University, and his Ph.D. (1965), from the University of Minnesota. He is a consultant to General Mills, Inc, Minneapolis. His research includes dispersed phase systems, stochastic modeling and applications, biocatalysis, and problems of general applied math interests.

Dhimakar S. Kompa is an assistant professor of chemical engineering at the University of Colorado. He received his B.Tech. from the Indian Institute of Technology, Madras and his M.S. (1982), and Ph.D. (1984) from Purdue University for his studies on bacterial growth on multiple substrates. His current research interests include kinetics and genetic instability of recombinant microbial populations, fermentations of hydrocarbons and mammalian cells, secondary metabolism, and affinity separations of proteins and cells.

George T. Tsao is a professor of chemical engineering and the director of the laboratory of renewable resources engineering at Purdue University. He received his M.S. (1986) from the University of Florida and Ph.D. (1960) from University of Michigan. He is the editor of Annual Reports on Fermentation Processes, his research interests are in the areas of biocatalytic engineering, food, fermentation, and enzyme technology.