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Predictive microbiology: uses for assessing quality and safety of dairy products

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SUMMARY

Predictive growth models can be used to predict the shelf-life of products at any point from processing to purchase and to assist manufacturers devise suitable preservation strategies that inhibit the growth of spoilage and pathogenic organisms to an acceptable extent. A number of spoilage models for raw and pasteurized milk and cottage cheese are presented. In most fermented dairy products, the vigorous metabolic activity of starter cultures prevents the growth of pathogens which limits the value of predictive models. Such modeling does however have application, particularly with respect to temperature abuse, in products such as pasteurized milk and cream where the perceived 'naturalness' of the product prevents the use of chemical preservatives. Two models describing the growth of *Listeria monocytogenes* are presented. It must be remembered that it is not sufficient to observe that a model appears to work: it is important to know why it works which emphasizes the need for continued fundamental studies on the physiology of microorganisms to underpin the more applied aspects of modeling research.

INTRODUCTION

Predictive growth modeling has the potential to allow manufacturers to predict the shelf-life and safety of milk and dairy products at the design stage, thereby reducing the need for ad hoc microbiological examination of new food products. Such models would also allow manufacturers to optimize quality and safety in food production and distribution chains and aid day-to-day decision making. All of these would contribute towards the implementation of suitable Hazard Analysis of Critical Control Point (HACCP) schemes for the dairy industry.

Although the potential advantages of using microbial growth models are significant, to the author's knowledge few models exist that can be applied directly to dairy products. Much of the effort has been directed to defining those conditions that limit microbial growth which has resulted in the ready availability of tables of minimum values for key spoilage and pathogenic bacteria. However, much of those data were generated when other factors such as pH and redox potential were near optimal. The data are therefore unrealistic since the concept of 'compensation' would be operating. This enables an organism to tolerate extremes of one parameter, a low pH for example, when other parameters such as temperature and concentration of nutrients are near optimal. It also takes no account of synergistic effects between preservation treatments. Whereas it can be argued that the existence of compensation and synergistic effects between preservative treatments builds in, if anything, a safety margin the extent of that margin remains unknown. This can ultimately lead to complacency regarding the true shelf-life and safety of foods. In addition, increasingly stable and safe food products are being recognized to be the consequence of preservative factors acting in combination often at levels which singly would not be inhibitory. There is a need therefore for validated predictive growth models for the main spoilage and pathogenic microorganisms in the full range of milk and dairy products.

Predictive growth models can be used in two main ways, firstly to predict the remaining shelf-life, at a reference temperature, of a food product at any point from processing to purchase if the temperature history is known. Secondly, to devise a preservation strategy that inhibits the growth of spoilage and pathogenic organisms to an acceptable extent.

With regard to spoilage four conditions must be satisfied before the shelf-life of a product can be predicted viz:

(1) The number of spoilage organisms in the product is known after processing.
(2) A mathematical equation has been devised which accurately describes the growth of the spoilage organisms at a range of temperatures likely to be encountered and

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which takes into account the other intrinsic and extrinsic preservation parameters which apply.

(3) The number of spoilage organisms is known when spoilage is apparent.

(4) The spoilage of the food must be as a result of the growth of organisms after the product has been processed.

It is, however, the case with some dairy products that spoilage can be the result of the action of enzymes which originate from psychrotrophic bacteria killed on pasteurization [27]. These enzymes, particularly proteases and lipases, can however survive pasteurization and even UHT heat treatments causing defects in those dairy products stored for comparatively long periods before consumption, such as UHT milk, cheese and butter [11]. In these instances modeling the growth of microorganisms after pasteurization would not predict spoilage. It is perhaps for this reason that spoilage models have been restricted mainly to raw and pasteurized market milk and cottage cheese.

SPOILAGE MODEL FOR RAW MILK

Muir and Phillips [32] examined silo and ex-farm bulk tank milk, the latter from a single source, and determined the generation times of the gross psychrotrophic microflora at 4, 6 and 8 °C. They found a wider variation in generation time among psychrotrophs in silo milk compared to bulk tank milk as might be expected from the more ubiquitous origin of contaminants in the former. The generation times at 4, 6 and 8 °C for both types of milk (150 samples in total) were pooled and using a rejection threshold of 5×10^6 psychrotrophs ml^{-1}, obtained from consulting the literature [28,29], generation times were calculated for a range of storage times and initial counts by insertion into a simple growth equation (Eqn 1), taken from Muir and Phillips [32].

$$\text{Equivalent generation time} = \frac{0.301 \times \text{storage time (h)}}{\log_{10}(\text{final count}) - \log_{10}(\text{initial count})} \quad (1)$$

The probability of those generation times occurring in the normal psychrotrophic microflora was determined and this information was used to construct a table of safe storage times for refrigerated raw milk at different temperatures (Table 1). Muir and Phillips [32] suggested that if the probability of the count exceeding 5×10^6 psychrotrophs ml^{-1} during storage exceeded 10% then this represented an unacceptably high risk in creamery operations. Obviously, if the raw milk is destined for a product particularly sensitive to proteolytic or lipolytic spoilage then this probability threshold can be lowered. Current methods available for the selective enumeration of psychrotrophs require an overnight incubation or longer which reduces the value of this predictive model. Methods do exist for the rapid (less than 30 min) determination of total viable counts such as bioluminescence [4] and the Direct Epifluorescent Filter Technique [36] but these are not selective for psychrotrophic bacteria and it remains to be determined to what extent the results of such rapid methods can be applied to the probability table (Table 1). In addition, this model does not have the advantage of predictive models now being developed which can take into account fluctuations in temperature. However this work has highlighted the need for good cleaning regimes to minimize psychrotroph contamination and the importance of maintaining proper refrigeration, since small differences in temperature can have a significant effect. These two factors should therefore be regarded as Critical Control Points (CCPs) in dairy processing operations.

TABLE 1

Probability values for 'safe' storage of refrigerated raw milk

Storage period (h)	Initial count	Generation time (h)	Probability of count exceeding 5×10^6 CFU ml^{-1}		
			4 °C	6 °C	8 °C
24	50 000	3.61	0	0	0
	100 000	4.25	0	0	0
	150 000	4.74	0	0	0.03
	200 000	5.17	0	0.02	0.08
	250 000	5.55	0.03	0.04	0.15[a]
48	5 000	4.80	0	0	0.05
	15 000	5.73	0.03	0.07	0.20[a]
	25 000	6.28	0.06	0.15[a]	0.34[a]
	50 000	7.22	0.17[a]	0.31[a]	0.64[a]
	100 000	8.50	0.34[a]	0.65[a]	0.84[a]
72	250	5.04	0	0.02	0.07
	500	5.42	0.02	0.03	0.14[a]
	1 000	5.86	0.05	0.09	0.21[a]
	2 000	6.38	0.07	0.16[a]	0.36[a]
	5 000	7.22	0.17[a]	0.31[a]	0.64[a]

[a]Probability of count exceeding 5×10^6 CFU ml^{-1} greater than 10%. Taken from Muir and Phillips [32].

SPOILAGE MODELS FOR PASTEURIZED MILK

The main causative organisms responsible for the spoilage of pasteurized milk are psychrotrophs [22] which gain access as post-pasteurization contaminants [20]. The actual spoilage phenomenon is therefore probably due to the hydrolytic action of proteases and lipases elaborated by these organisms. A number of models have been proposed for this product.

Pasteurized milk – spoilage model 1

Predictive growth models for homogenized whole milk incorporating a range of organisms isolated from the product have been devised by Langeveld and Cuperus [26]. These authors used the Arrhenius equation (Eqn 2)

$$\ln k = C - u/RT \quad (2)$$

where C = a constant, u = temperature characteristic ($J\ mol^{-1}$), R = gas constant (8.32 J mol $°K^{-1}$) and T = temperature (°K).

The necessary 'u' value can be calculated when the Arrhenius plot (ln k plotted against the reciprocal of the temperature, $1/T$) approximates to a straight line. However, when the authors subjected their data to such a plot, convex curves were observed with the curvature being particularly pronounced at temperatures above 30 °C and below 10 °C. This non-linearity when bacterial growth data is subjected to an Arrhenius plot has been observed by other workers [37] and severely limits the usefulness of the model particularly at the refrigeration temperatures under which pasteurized milk is usually stored.

Pasteurized milk – spoilage model 2

Janzen et al. [24] stored raw milk at 4.5 °C for 0, 2, 4 and 6 days prior to pasteurization with subsequent storage also at 4.5 °C. The milk was analyzed initially prior to pasteurization and after heat treatment at 4-day intervals for up to 20 days for numbers of coliforms, psychrotrophs, mesophiles and flavor (ADSA). A significant decrease ($P<0.01$) in initial flavor score of the pasteurized product was observed as storage time of raw milk increased. The age of the raw milk was also found to have significant ($P<0.01$) linear effect on the shelf-life of the resulting pasteurized product. This enabled a predictive equation (Eqn 3, taken from Jansen et al. [24]) to be devised relating flavor score and shelf-life of pasteurized milk to the age of raw milk.

$$\text{Flavor score} = 8.00 - 0.088R - 0.11P - 0.0015P^2 - 0.009RP \quad (3)$$

where R = days held raw at 4.5 °C and P = days stored pasteurized at 4.5 °C.

Using this equation the shelf-life of the pasteurized product can be calculated by setting a minimum acceptable flavor score (usually 6.0) and substituting the days held raw at 4.5 °C.

It is interesting to note that the raw milk used was of very high quality with total bacterial counts of less than 1000 CFU ml^{-1} and the number of psychrotrophs in the raw milk never reached 5×10^6 CFU ml^{-1} at which defects due to these organisms can be expected [32]. The fact that the age of the raw milk not only affected the shelf-life of the pasteurized product but also its initial flavor score indicates that the observed relationship may reflect not only a low level of post-pasteurization contamination but also the action of intrinsic bovine hydrolytic enzymes such as proteases and lipases since there is evidence that they can survive pasteurization [12,13].

The robustness of the prediction equation (Eqn 3) both in terms of ability to accommodate a range of storage temperatures and raw milk samples of lower quality have to be determined before it can hope to have widespread application.

Pasteurized milk – spoilage model 3

Griffiths and Phillips [18] using a combination of preincubation of the pasteurized milk sample followed by viable plate counts on a range of selective media, devised both simple and complex equations to predict shelf-life at any required temperature. Since there was little difference in the predictive accuracy of these equations only the simplified version is shown (Eqn 4, taken from Griffiths and Phillips [18]).

$$\text{Shelf-life (h)} = \frac{1}{[0.00621\{T - 269.55 - 0.74(CFC_{15}) - 0.11(CFC_{15})^2\}]^2} \quad (4)$$

where T = storage temperature in °K and $CFC_{15} = \log_{10}$ count after preincubation of pasteurized milk for 25 h at 15 °C and enumeration on milk agar (25 h at 21 °C) containing CFC supplement (Oxoid).

The fact that a preincubation stage of 25 h followed by a viable count requiring a further 25 h means that the results can only be used retrospectively. In addition the equation cannot accommodate fluctuations in storage temperature.

Pasteurized milk – spoilage model 4

Bishop and White [2] evaluated protease activity, endotoxin concentration and impedance detection time in addition to viable plate counts as indicators of potential shelf-life at 7 °C determined solely by organoleptic assessment. Of all these methods the use of a preincubation period of the milk at 21 °C for 14 h followed by impedance detection proved to be the most reliable predictor of shelf-life. An equation (Eqn 5, taken from Bishop and White [2]) was generated relating Impedance Detection Time (IDT) with shelf-life.

$$\text{Shelf-life (days)} = 0.560 + 1.400\,(\text{IDT}) - 0.032\,(\text{IDT})^2 \quad (5)$$

$r = 0.93$

In general these authors found that an IDT <6 h indicated a potential shelf-life at 7 °C of ≤9 days while an IDT >9 h indicated a shelf-life of ≥11 days at 7 °C.

For this range of milk quality the time required for a result would be 14–23 h assuming that with poor quality milk an impedance change was detected immediately after the necessary 14 h incubation at 21 °C. Although the time required to complete the test is less than the method of Griffiths and Phillips [18] (Pasteurized milk – spoilage model 3) a result is still not obtained within the working day and the model takes no account of storage temperature fluctuations both of which reduce the value of this predictive test. The high cost of an impedance detector is also prohibitive.

It is interesting to note however that impedance changes are not only caused by bacterial metabolism per se, but also the action of extracellular enzymes such as proteases and lipases. This may explain why impedance detection time was the best predictor evaluated.

Pasteurized milk – spoilage model 5

Chandler and McMeekin [9] investigated the relationship between storage temperature and rate of deterioration of

pasteurized homogenized milk. At temperatures up to 12 °C psychrotrophs, the main spoilage agents, exhibited a growth response to temperature which could be adequately described by a relative rate function based on the Square root model of Ratkowsky et al. [38] as shown in Eqn 6.

$$\sqrt{r} = b\,(T - T_{\min}) \tag{6}$$

where r = rate of deterioration of the product, T = temperature of storage (°K), $T_{\min}$ = notional minimum temperature for growth (for psychrotrophs = 263 °K) and b = a constant.

The $T_{\min}$ value appears to be an intrinsic characteristic of an organism since it was not found to vary significantly when bacteria of dairy origin, with the exception of *Bacillus circulans*, were grown in pure or mixed cultures on a range of milk substrates.

The authors incorporated the relative rate function into an electronic device (Time/Temperature Function Integrator) which was used to monitor the temperature history of pasteurized milk, displaying the integrated information as an equivalent number of days at a reference temperature. This represented the shelf-life that had expired to date. In order to calculate the remaining shelf-life, again at a reference temperature, further information is required, namely the number of psychrotrophs present when spoilage is detectable organoleptically and the initial level of psychrotroph contamination. Chandler and McMeekin [9] used log (7.5) bacteria ml^{-1} as suggested by Griffiths et al. [19] to represent the end of shelf-life. The initial level of psychrotroph contamination represented more of a problem since, to this author's knowledge, no method is currently available which can rapidly (<15 min) and selectively detect low psychrotroph numbers. Chandler and McMeekin [9] suggested an initial psychrotroph contamination level of 10 psychrotrophs ml^{-1} which they considered was achievable by good manufacturing practice and which would allow an acceptable shelf-life.

There are a number of limitations to this approach. Firstly, even a comparatively small variation in the initial level of psychrotroph contamination can have a significant effect on shelf-life (Table 2). This is compounded by the fact that even within one batch of pasteurized milk there is a wide variation in microbial load within sample units [31, 47]. However, this does represent a testing regime which has a worthwhile and practical application since milk processors can incubate milk at 12 °C which maximizes the rate of psychrotroph spoilage [9] and determine, using organoleptic assessment alone, the actual shelf-life. A shelf-life of less than 3.2 days indicates a problem with post-pasteurization contamination while a shelf-life greater than 3.2 days incidates good manufacturing practice.

The problem of variation in quality among sample units may be partially overcome by the use of a three-class sampling plan which takes more account of the non-random distribution of organisms among sample units [44].

TABLE 2

Shelf-life prediction for pasteurized milk

Temperature (°C)	Relative rate of spoilage	Initial number of psychrotrophs ml^{-1} Days to reach spoilage		
		1	10	100
0	1.00	19.2	16.7	14.2
2	1.44	13.3	11.6	9.8
4	1.96	9.8	8.5	7.2
6	2.56	7.5	6.5	5.5
8	3.24	5.6	5.2	4.4
10	4.00	4.8	4.2	3.6
13	5.29	3.6	3.2	2.7
16	6.76	2.8	2.5	2.1

Figures for days to reach spoilage assume a generation time of 8 h at 5 °C and spoilage at log 7.5 psychrotrophs ml^{-1}. Taken from Chandler and McKeekin [8].

Pasteurized milk – spoilage model 6

An enzyme/chemical-based indicator which changes color according to its cumulative time and temperature exposure was used successfully by Grisius et al. [21] to estimate the total viable mesophilic population in pasteurized milk during storage. A similar time-temperature indicator which exhibited a delay prior to initiating a discernible response was found suitable for estimating the time delay before germination of psychrotrophic *Bacillus* spores which have been implicated in the spoilage of pasteurized milk [35].

Such indicators are cheaper than the temperature function integrators of Chandler and McMeekin [9] and offer the potential to be applied to all pasteurized milk containers thereby taking into account the differing storage regimes applied to sample units within a batch. It is unlikely however that a single indicator would be able to account for the differences in the type of contaminants in milk from different processors or describe the complexity of microbial growth patterns arising from mixed populations incubated at fluctuating temperatures [21]. This is less of a problem for electronic integrators which can accommodate a composite series of growth curves [8].

SPOILAGE MODELS FOR COTTAGE CHEESE

Cottage cheese – spoilage model 1

Bishop and White [3] using a similar approach to that which they employed with pasteurized milk [2] evaluated impedance detection time, plate counts, endotoxin (lipopolysaccharide) concentration and proteolysis as predictors of cottage cheese quality. Using a formal organoleptic assessment to detect the end of shelf-life, these authors found that plate counts of mesophiles, psychrotrophs and Gram-negative bacteria were of little value whereas endotoxin concentration, degree of proteolysis and particularly impedance detection time could provide an acceptable estimate of shelf-life. From a practical point of view at creamery level

perhaps the best predictor to employ, at least initially, is endotoxin analysis since it is rapid, comparatively simple to perform and requires no expensive or sophisticated equipment.

Cottage cheese – spoilage model 2

Shellhammer and Singh [43] carried out an experiment to determine efficacy of two types of enzyme-based time/temperature indicators (Models #4014 and #4021; I-Point Biotechnologies AB, Nya Agnesfridsvagen 181, S-213 75 Malmo, Sweden) to monitor the quality of cottage cheese. The cottage cheese samples were stored at 2.7, 8.8, 15.1 °C and at a variable temperature condition where the temperature was chiefly held at 3 °C for a period (7 h) and then raised as high as 30 °C. A number of quality attributes were determined viz, mesophile and psychrotroph counts, pH value and titratable acidity. Two different spoilage microflora were observed with psychrotrophs dominating at 2.7 °C and the varied temperature condition while lactic acid bacteria predominated at 8.8 and 15.1 °C. After calculation of relevant regression equations it was shown that three of the quality attributes viz mesophile count, pH value and titratable acidity could be predicted by model #4014. Such relationships were not found for model #4021.

The results indicate that with cottage cheese no one indicator would be able to effectively predict growth of two groups of microorganisms under all the likely temperature conditions. This suggests that the enzymatic temperature history devices would have the greatest value as indicators of temperature abuse.

SAFETY OF MILK AND MILK PRODUCTS

In most fermented dairy products the metabolic activity of the starter cultures employed prevents the growth of pathogens through the production of some or all of the following inhibitory agents: lactic acid, acetic acid, hydrogen peroxide and bacteriocins e.g. nisin [10]. Other factors such as the use of salt and low water activity and oxygen tension may also contribute to this inhibition.

Pathogens in general, if they survive the starter culture fermentation, remain inactive or decline during ripening [14, 23,42] although in some cases multiplication has been observed at the beginning of the ripening period [40]. Problems can arise, however, during the fermentation stage if the starter culture is not vigorous enough as a result of phage attack or contamination of milk with antibiotics [10]. Under these conditions organisms such as *Staphylococcus aureus* may produce enterotoxin that can survive into the product [34,45] or other pathogens may multiply to such an extent that even at the end of the ripening period, viable numbers are still greater than or equal to the requisite infective dose. This is particularly important when raw milk is used. This problem is best addressed by the adoption of good manufacturing practice rather than modeling techniques. Models could however be generated to maximize the rate of decline of pathogens in such products to ensure that, given a realistic level of initial pathogen contamination, all pathogens have been killed at a stage when the product has reached its optimal organoleptic state. With some of the more ubiquitous pathogens, such as *Listeria monocytogenes*, this approach may be worthwhile as long as it does not detract from the importance of good hygienic practice. There are some fermented dairy products however such as Brie and Camembert cheese where the pH value increases significantly during ripening or Mexican style soft cheeses where acid development is minimal. *Listeria* will grow in such products unless some other controlling factor such as salt is used e.g. Stilton (Table 3). Predictive growth modeling clearly does have application in such foods and in non-fermented dairy products e.g. dairy desserts and milkshakes. For products such as pasteurized milk and cream where the perceived 'naturalness' of the product prevents the use of chemical preservation regimes, modeling growth of pathogens with respect to temperature abuse in production and distribution chains is of particular interest.

One such model has been devised by Murphy et al. [33] for one strain of *L. monocytogenes* using 10% w/v reconstituted skim milk. Three parameters were modeled viz temperature (3–35 °C), pH (4.5–6.5) and salt concentration (4.5–6.5% w/v). An interactive effect was observed between pH and temperature since at low pH values growth increased with temperature while at low temperatures only a marginal increase in growth occurred as the pH approached neutrality from its lower limit. Independent data from the same experimental system was found to fit the model with r^2 values of 96% and 84% being achieved when the exponential growth rate and lag phase duration respectively were used for the comparison. This however cannot be considered a fully validated model since pure cultures of the pathogen were used thus eliminating any interactive effects exerted by the remaining microflora.

Buchanan and Phillips [6] developed a response surface model to describe the interactions of temperature (5–37 °C), pH (4.5–7.5), salt (5–45 g L^{-1}), sodium nitrite (0–1000 μg L^{-1}) and atmosphere (aerobic vs anaerobic) on the growth of pure cultures of *L. monocytogenes* in Tryptose Phosphate Broth. When predicted values were compared with published growth kinetics data for the pathogen in whole milk and 2% milk the model consistently underestimated the generation time and overestimated the lag phase duration

TABLE 3

Preservation factors for cheese

	pH		Salt in moisture	Water activity	Moisture
	curd	mature	% w/w	a_w	% w/w
Cheddar	5.0	5.2–5.4	4.6	0.97	39
Stilton	4.5	6.0–6.5	5.9	0.96	42
Brie	4.8	6.5	3.4	0.98	52
Cottage cheese	4.7	4.9	1.9	0.99	80
Mexican soft	6.3	6.2	3.3	0.98	52

Taken from Jervis [25].

though not to an extent that they could not be considered as 'first round estimates'. This model is particularly useful because it lends itself to the development of user-friendly software. It would however not be considered to represent a worst case scenario since preincubation of milk with pseudomonads has been shown to significantly ($P<0.05$) enhance the growth of *L. monocytogenes* [30].

To the author's knowledge few fully validated models have been published for the growth of pathogens in dairy products. There is however much published work on the growth or survival of pathogens such as *L. monocytogenes* in products like Camembert cheese [39], butter [15] and cold pack cheese [41], but in these instances there is insufficient data to construct models which can accommodate fluctuations in temperature and variations in the level of appropriate preservative factors e.g. salt concentration, pH value and water activity for any one dairy product. These data are however important as they indicate the products where there is a high risk of survival or growth of pathogens and therefore where initial effort should be concentrated. They also indicate the preservative factors which should be taken into account in the design of experiments directed at the generation of models.

There are numerous published papers on the growth of pathogens using broth systems e.g. *Staphylococcus aureus* [1], enteropathogenic *Escherichia coli* [16], *Shigella flexneri* [48], *Salmonella typhimurium* [46], *L. monocytogenes* [7] and *Clostridium perfringens* [17] among others. It remains to be determined, however, to what extent growth responses of organisms in such broth systems mimic growth of the same organisms in real foods. As data and experience accumulate it will become clear which foods mimic growth responses in broth, but until that time it is highly desirable that models be validated before reliance is placed on them.

PROSPECTS FOR FUTURE RESEARCH

It is clear that because of the 'perceived' naturalness of most non-fermented dairy products there is reduced scope for modeling research on safety aspects except in those products such as dairy desserts and milkshakes in which a range of preservative treatments can be used. Modeling of the spoilage of these products viz raw milk, pasteurized milk and cottage cheese, however has been addressed and useful models currently exist. With regard to the safety of fermented dairy products the modeling approach would be best directed at those products which have a high pH value either throughout or at the end of the ripening period e.g. Mexican style soft cheese and Camembert respectively. With other fermented dairy products in which vigorous fermentation by starter cultures occurs then modeling could be applied to ensure that the number of viable pathogens have declined to a level that is below the infective dose before the end of the ripening period. Modeling of the spoilage of fermented dairy products would be complex because of the contribution of heat-stable enzymes (proteases and lipases) which are carried over into the product as a result of the prior growth of psychrotrophs in raw milk.

Modeling research, whether concerned with safety or spoilage aspects, will undoubtedly reveal synergistic effects between preservative treatments giving insights into the physiology of particular organisms which will prove worthy of further investigation. In addition, it must be remembered that it is not sufficient to observe that a particular method or model appears to work: it is important to know why it works, otherwise there is no means of knowing when and how it will not [5]. This emphasizes the importance of continued fundamental research on the physiology of microorganisms to underpin the more applied aspects of modeling research.

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