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Scale-up of Heat Sterilization Operations

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Sterilization of a fermentation medium is usually accomplished in a cycle that includes heating the medium to some prescribed so-called sterilization temperature, holding it there for a specified period of time, and cooling it to process temperature. The productive capacity of most media is adversely affected by prolonged heating due to degradation of some nutrients or the production of substances which are toxic to the microorganisms used in the subsequent fermentations. In many cases, therefore, media sterilization presents two incompatible objectives. On the one hand, it is desired to maximize contaminant destruction, while on the other, damage to the nutrient quality of the medium must be minimized.

Although continuous sterilization offers inherent advantages with regard to the objectives of a sterilization operation, very little work has been published describing in just what manner these objectives can be optimized, or even translated in process scale-up. Design is based largely on achieving satisfactory contaminant destruction with little concern other than one of conveniently trying to minimize the heat exposure. Contaminant destruction calculations can be carried out quantitatively but damage to nutrient quality has usually been examined only in a qualitative light. The same remarks, of course, also apply to batch sterilization.

This paper briefly examines quantitative changes in both contaminant destruction and damage to nutrient quality in a medium as scale of operation is changed. A quantitative basis for nutrient quality damage rests on the fact that adverse changes which occur in the medium during sterilization are chemical reactions...
which in many cases can be described kinetically by chemical reaction rate equations. Based on chemical observations a quality criterion can be defined. This criterion is analogous to the sterilization criterion described by Deindoerfer and Humphrey (1959), since it characterizes nutrient quality destruction in the same manner in which the sterilization criterion characterizes contaminant destruction. Reproducibility of process yields in scaling-up depends upon the proper assessment and translation of both of these criteria. The use of a simple graphical method employing a process performance chart, which characterizes these criteria as functions of the sterilization conditions, is suggested.

Considerations in Scaling-Up

In any sterilization, the initial total number of viable spores is equal to \( C_1V \) where \( C_1 \) is the viable spore concentration in the raw medium and \( V \) is the volume of the raw medium. Although sterility is an absolute condition, it should be clearly evident that its approach as accomplished by heat treatment is a probabilistic one. The chance of successful attainment of an absolutely sterile condition depends on how small a finite number of spores remain viable after sterilization. By design this number is set equal to \( P \) where \( P \) is the probability of a single spore in volume \( V \) surviving the heat treatment. Thus, if there is to be a chance of 1 in 100 of the sterilization failing, \( P \) would be equal to 0.01. The sterilization criterion can then be written

\[
v = \ln \frac{C_1V}{P}
\]  

(1)

It should be recognized that the least number of viable spores necessary to contaminate any quantity of medium, regardless of its volume, is 1. If the same chance of successful attainment of absolute sterility is to be maintained as larger volumes are sterilized, the severity of the heat treatment must be increased. This is probably the most over-looked point in sterilization scale-up! The achievement of a similarly sterile condition, therefore, is scale dependent. For every 10-fold increase in operational scale, the sterilization criterion must be increased by 2.3. Sterilization similarity for a given

![Figure 1. Effect of sterilization time on streptomycin yield](http://aem.asm.org/)

DATA OF TANNER, VOJNOVICH & VAN LANEN (1949)
medium, then, is represented by the following expression, where subscripts \( L \) and \( S \) refer to large and small scale, respectively.

\[
v_L = v_S + \ln \frac{V_L}{V_S}
\]  

(2)

The Nutrient Quality Criterion

In fermentation processes where yield is insensitive to the sterilization operation, obviously only one similarity condition, namely, that of a similarly sterile condition, is necessary for scale-up. In many processes, however, heat treatment of the medium has a marked effect on process yield. In these cases an additional condition must be satisfied, namely, similarity in the nutritive quality of the medium.

Two examples of reduced process yields due to the effects of prolonged heat treatment during sterilization are illustrated in figures 1 and 2. The first figure shows the effect of prolonged heating on streptomycin yield as determined by W. H. Bartholomew and E. O. Karow (Personal Communication, 1959) during some very early investigations of the streptomycin fermentation process. The original soybean meal medium in which strain comparisons were carried out apparently degraded only slowly on prolonged sterilization. The productive capacity of an improved medium in which a higher producing mutant strain was tested, on the other hand, was greatly decreased by excessive sterilization. An interesting observation to make is that in batch sterilizations carried out in large fermentors,
where long heating and cooling portions of the sterilization cycle occur, the improved medium will not show its full potential and, very likely, will not be any better than the original medium. Thus, its value could very well go undetected. This is an ideal situation in which to employ continuous sterilization.

Figure 2 illustrates the deleterious effect of long sterilization on riboflavin yield as reported by Tanner, Vojnovich, and Van Lanen (1949). The two curves show clearly that yield is reduced considerably after 30 min of heat exposure. This is usually the time employed in most batch sterilizations for media of the type used in the riboflavin fermentation. Notice the maximal and minimal plateaus and the intermediate transition range. The shapes of these curves lend strong support to the idea of destruction of an essential nutrient, and attention will be called to them again later.

A number of undesirable chemical changes in nutrient medium during heat treatment have been identified. They are mostly reactions that are either bimolecular where one species is in large excess, for example, the reactions of certain amino acids with sugars; or monomolecular, such as the degradation of vitamins and other essential growth factors. The reactions in these cases approximate first order kinetics, and the equations such as developed for expressing the sterilization criterion can also be used for calculating nutrient degradation.

The dependence of yield on nutrient is not always a simple one, but it is quite evident. Figure 3 for example, illustrates the dependence of ultimate yeast yields on various heat labile growth factors as demonstrated by White (1954). Three characteristics should be noticed in each plot. There is a minimal yield value or minimal process performance corresponding to no growth factor. There is a maximal concentration above which there is no further improvement in performance. In the range below the maximum, or critical concentration, performance varies widely with concentration. Now, recall the riboflavin medium sterilization data shown in figure 2. On prolonged heating, growth factors such as these are destroyed almost completely, resulting in a minimal yield. For short sterilization, however, yield was relatively insensitive, indicating that the concentrations of various essential growth factors remain above their critical levels. For an intermediate period of time, there is a large change in yield as the concentration of growth factors is reduced from critical to minimal levels.
Comparison of Sterilization and Nutrient Quality Criteria

The molecular rearrangements involved in undesirable chemical reactions are thermodynamically more easily accomplished than thermal destruction of spores. This means that the ratio of the rates of chemical degradation as compared to those for spore destruction decrease with increased temperature.

Another important difference lies in the fact that the sterilization criterion depends on the number of surviving spores, i.e., the value assigned to \( P \) in equation (1), for a particular volume. It is, therefore, scale dependent. The nutrient quality criterion, \( \Lambda \), on the other hand, depends on the poststerilization concentration of an essential nutrient.

\[
\Lambda = \ln \frac{C_1}{C_2}
\]

(3)

Here \( C_1 \) and \( C_2 \) are the pre- and poststerilization concentrations of essential nutrient. No volume factor appears in this equation and thus, the nutrient quality criterion is independent of scale.

Scale-up Method

For proper scale-up of a sterilization operation where process yield varies with sterilization conditions, it is necessary that both sterilization and quality similarity must be maintained.

Figure 4 shows a process performance chart for a hypothetical sterilization carried out in a steam injector, flash cooler sterilizer. The basic concept of this chart stems from the thermal process charts sometimes used in the food industry, first suggested for use in fermentation studies by Finn (1953). Although overly simplified in this example, the chart illustrates some very important points concerning sterilization scale-up.

Two sets or families of lines appear on the chart. Each line in the steeper sloped set represents a locus of sterilization conditions which yield the same sterilization end point, i.e., the severity of the heat treatment from a sterilization point of view is identical along any one line. It has already been shown that as scale of operation increases, the severity of the heat treatment must increase if the same sterilization end point is desired. An increase in scale, and thus sterilization severity, is indicated by the direction of the dashed arrow in the lower left hand portion of the chart.

The second set of lines represent the loci of sterilization conditions which result in various poststerilization concentrations of an essential nutrient. As this concentration is maintained at higher levels, within certain limits, as shown by the dashed arrow in the upper right hand portion of the chart, an increase in process yield is achieved. These lines represent, in essence, constant process performance lines. The two broken lines in this set signify two important limits. The upper line is the yield corresponding to a negligible amount of essential nutrient. The lower one represents the yield corresponding to the critical nutrient concentration, above which no further increase in yield can be expected. These are the minimal and maximal process performance lines, in so far as yield is affected by sterilization.

The maximal and minimal lines divide the chart into three regions. The upper region is the minimal yield region. An arbitrary change in scale in this region has no effect on process yield because the quality of the medium has been completely degraded. The lower region is the safe operating region. A change in scale here again has no effect on process yield, as fortunately the critical essential nutrient concentration is exceeded at all times so that the maximal process yield is always attainable. In the transition range between these lines, however, it is seen that performance can be affected by an indiscriminate scale-up.

Examining the point in the transition region from which a move is indicated in three directions, it is shown that an increase in either time, or temperature, will result in a decreased yield. However, moving along a constant yield line assures a reproducible process. This brings to light an important corollary: in scaling-up a sterilization operation, sterilization time should be decreased and sterilization temperature increased. Only in this way can similar process performance be achieved.

All sterilizations, of course, are not treated as simply as one in a steam injector, flash cooler sterilizer from the design viewpoint. In other types of equipment the
heating and cooling portions of a sterilization cycle must be taken into account. Also, sterilization of most fermentation media undoubtedly causes a number of undesirable chemical changes, and sometimes even beneficial ones, and usually involves more than one essential nutrient. A proper experimental examination of the time-temperature effects on process yield should, nevertheless, permit the construction of a process performance chart for any process with a function of time at so-called sterilization temperature as the ordinate and a function of so-called sterilization temperature as the abscissa. The loci of the two similarity criteria in such situations would be curved lines. Interpretation of the chart would be no different, however, than for the simple case outlined, and useful scale-up information would be provided.

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Influence of Sorbic Acid on Populations and Species of Yeasts Occurring in Cucumber Fermentations

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In the natural fermentation of brined cucumbers a part of the subsurface microbial activity is due to yeasts. Active growth by these organisms in commercial cucumber brines was first reported in 1941 (Etchells). Subsequent work on this phase of the fermentation has included studies directed toward establishing the identity of the individual species comprising the total yeast population in brines. Information of this type has been reported for two major cucumber brining areas in the country—northern and southern (Etchells and Bell, 1950; Etchells, Costilow, and Bell, 1952). The findings of these two studies, based on the identity of nearly 1900 cultures of yeasts isolated from brines during various stages of fermentation, revealed that the pattern for the principal yeast species in brines from both brining areas was very similar. Seven of the nine species found were obtained from both northern and southern brines.

During the brining seasons of 1954, 1955, and 1956 we had an opportunity to extend that portion of the work related to the southern brining areas while studying the microbial flora in experimental cucumber brines to which sorbic acid (2,4-hexadienoic acid) or its sodium salt had been added. This fungistat was introduced into the brine in an attempt to control yeasts and thus prevent the formation of "bloaters" (hollow cucumbers) caused by gaseous fermentation (Jones et al., 1941). A number of yeasts were isolated from brines both with and without sorbic acid. It is our purpose in this paper to list the species isolated under the various experimental conditions used and to compare these findings with those previously reported from this laboratory.

MATERIALS AND METHODS

The cucumbers were brined in wooden vats each containing approximately 30 bushels of freshly harvested no. 3 size Model variety pickling stock. The vats were unsheltered and located under outside conditions at a commercial pickling plant in eastern North Carolina. Each vat, after being filled with cucumbers, representing a composite of the stock from two or more receiving stations, was fitted with a false, wooden head and salt brine of the desired concentration added to a level of a few inches above the head. Next, dry salt, in appropriate amounts, was added on the false head to maintain the desired initial brine concentration which otherwise would become diluted by the water content of the cucumbers. Sorbic acid or sodium sorbate was added to give the desired concentration by weight.