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Scale Down of Production Conditions in the Laboratory

Timothy C. Dodge*, Peter M.A. Lundqvist and Gopal K. Chotani

Genencor, A Danisco Division
925 Page Mill Rd, Palo Alto, CA 94304, USA
tim.dodge@danisco.com

Scale-up of bioprocesses from laboratory to production scale is a time-consuming and costly exercise. Time delays in process transfer and reduced process performance at production scale upon scale-up results in both lost sales and profits. However, adequately assessing large-scale conditions in the laboratory is a difficult task. Here we describe a method to simulate carbon source gradients in production-scale fed-batch bioreactors by pulse feeding of the substrate in laboratory bioreactors. The negative effect of carbon source gradients was found to be different for various processes, with some showing greater sensitivity than others. Solutions were suggested and when implemented at large scale resulted in process improvement bringing performance closer to laboratory results.

1. Introduction

Scale up of bioprocesses can be a lengthy and costly endeavor. Many aspects come into play, including, but not limited to, mixing, heat transfer, shear, and for aerobic cultures, oxygen transfer (Schmidt, 2005). Many of these cannot be optimized independently of one another. For example, mixing or blending can be increased through increased agitation. However, this can have negative effects on shear sensitive microorganisms or cell cultures. Oxygen transfer is positively influenced by increased power consumption. Radial flow impellers are an excellent means of transferring power into the broth. But such impellers lead to compartmentalized flow patterns and poor mixing. Conversely, axial flow impellers can give excellent bulk mixing, but generally are poor at dissipating power and can result in reduced oxygen transfer. Aerobic fermentations also tend to produce more heat by respiring cells, which also must be removed in order to maintain temperature. If cooling can be maintained through a jacket on the vessel, there is typically little effect upon mixing. But the addition of internal cooling elements required for some highly aerobic fermentations can negatively affect flow patterns and mixing.

A multitude of scale-up parameters and rules-of-thumb have been described, and can be used with varying degrees of success. But there are few methodologies for scaling down processes to the laboratory to understand the important effects on the culture. One such method has been to incorporate a tubular loop with the laboratory bioreactor to simulate the gradients in the stirred tank reactor (Papagianni et al, 2003). This method has the possibility of subjecting the culture to anaerobic conditions in the

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tubular loop. Here we describe another tool, the purpose of which is to evaluate substrate gradients in the fermentor, mainly coming from the use of fed-batch fermentation. Control of glucose feeding can have important impact on the performance of many processes. However, gradients found upon scale-up could have a detrimental impact upon the performance of the culture. This is due to microbial cells being able to respond to their environment in a manner of seconds. Laboratory vessels can match this characteristic time, while large vessels can have characteristic time one or two orders of magnitude larger (Lara et al, 2006). Pulsed-feeding of substrate to the laboratory fermentor was found to be a useful way of scaling down and simulating the gradients found at large scale. The effects of such gradients on a culture could be determined to better predict when there may be issues upon scale-up. In addition, when it was shown that substrate gradients were the primary cause of poor scale-up for a process, means to directly affect mixing in the large scale vessel were taken and the scale-up issue was nearly eliminated.

2. Scale-up for different industrial enzyme production processes

Industrial enzymes are produced by a number of different microbial cultures (Chotani et al, 2007). These are almost always aerobic cultures and optimal production results from using the fed-batch mode of fermentation. The feed component is typically the major carbon and energy source and is typically carbohydrate syrup. Process development is carried out in the laboratory with bench-scale reactors ranging in size from about 1 to as much as 50-L nominal volume. This may be followed by pilot plant development ranging in size from 0.1 to 5 m^3 nominal volume. But production scale is usually even larger ranging from 10 to 400 m^3 nominal volume.

Many processes scale up well without major loss in performance moving from the laboratory, through the pilot plant and into full scale manufacturing. However, there are processes where this is not the case. Performance decreases as the scale increases. Figure 1 shows the relative enzyme concentration normalized to the result in the laboratory fermentor. Maximum performance was achieved in the laboratory fermentor. And as the process was scaled-up to the pilot plant, enzyme concentration dropped by about 25%. But upon further scale-up to manufacturing equipment, enzyme concentration dropped even further to less than half of what was achieved in the laboratory. Sensitivity to factors such as temperature and dissolved oxygen had previously been studied and the results suggested that these would not result in the magnitude of reduction in performance observed at increased scale.

3. Pulse feeding to scale-down concentration gradients

A standard model for concentration gradients in large scale vessels is to assume that there are different zones, or compartments that are well mixed. The microorganisms present in the culture will circulate through the zones, experiencing different substrate

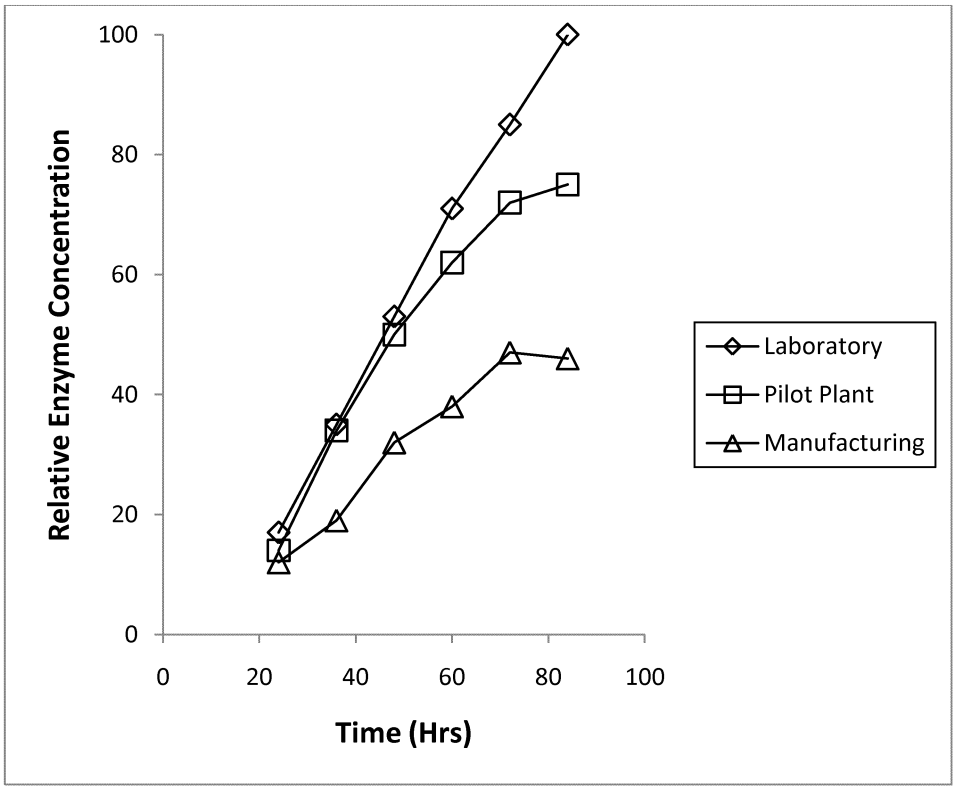

Figure 1: An example of poor industrial enzyme scale-up. Laboratory fermentation results in maximum enzyme production, which decreases as scale increases.

concentration in each of the zones. One model would be to assume two different parameters: 1) the size of the mixing zone for substrate feed as a fraction of the total volume, v_f, and 2) the circulation time in the vessel, t_c. The appropriate pulse feed rate, f_p, as a function of the desired continuous feed rate, f, would then be described as,

$$f_p = \frac{f}{v_f} \tag{1}$$

and the pulse feed time, t_p, as,

$$t_p = v_f \cdot t_c \tag{2}$$

For instance, one could assume that the substrate fed to the top of the fermentor is immediately well mixed in the top 20% of the volume, or v_f = 0.2 and that the mixing time were 2 minutes, or t_c = 120 seconds. Then the pulse feed protocol would be to feed continuously at 5 times the desired feed rate for 24 seconds and then no feed is added

for the next 96 seconds before starting the next pulse. Experiments were performed that indicated mixing time was the more sensitive of the two parameters until $v_f > 0.4$.

3.1 Pulse feeding results for the same industrial enzyme process

The same industrial enzyme process described in Figure 1 was performed again in the laboratory. This time, in addition to the experiment performed with continuous feeding, experiments were also performed with pulsed feeding. Results are shown in Figure 2 along with the same data from Figure 1. The feed parameters used for these experiments were continuous feed, pulsed feed with $v_f = 0.1$ and $t_c = 3$ minutes and pulsed feed with $v_f = 0.1$ and $t_c = 5$ minutes. The results of 3 and 5 minute pulsed feed in the laboratory fermentors were similar to what was observed for the pilot plant and manufacturing fermentors. One conclusion that can be drawn from this experiment is that the major contributor to the reduced performance upon scale-up is substrate gradients present in the larger vessels.

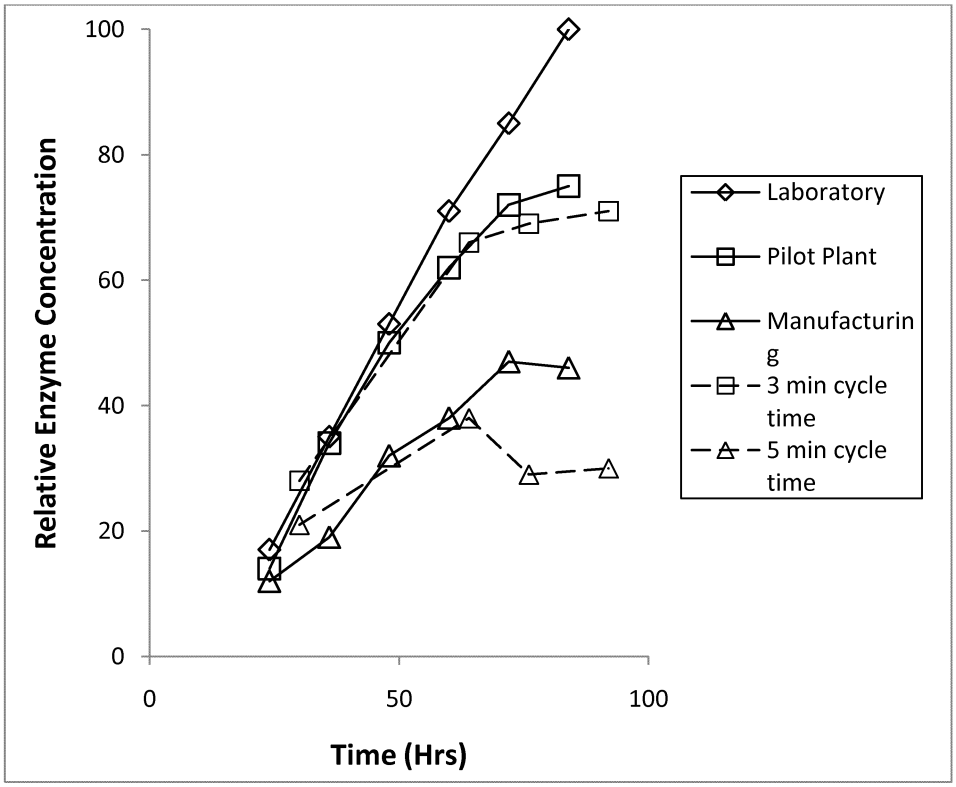

Figure 2: Pulsed feed of the same industrial enzyme process at laboratory scale. Continuous feeding results in maximum enzyme production, which decreases as pulsed feed mixing time increases.

3.2 Potential manufacturing vessel modifications

Pulse feeding experiments in the laboratory indicated that the substrate gradients, and thus mixing or blending, was the cause of poor scale-up of this industrial enzyme process. Similar pulse feeding experiments were performed with other processes as well. Some performed similarly to that shown above. However, there were also processes that were affected to a much lesser extent, if at all (data not shown). Nonetheless, substrate gradients and mixing were an issue at manufacturing scale and deserved attention.

Three methods of reducing mixing time in the manufacturing equipment were contemplated: 1) increasing the agitation rate, 2) replacing radial flow impellers with axial flow impellers, and 3) adding feed at additional points within the fermentor.

Increasing agitation rate might appear to be the simplest means of reducing mixing time, which is affected linearly by the agitation rate (Hadjiev *et al*, 2006). However, power draw required by the agitator motor is proportional to the agitation rate to the third power (Schmidt, 2005). This means that cutting the mixing time in half by increasing the agitation rate by 2x would require 8x motor power.

The manufacturing fermentor was outfitted with three radial flow impellers. These function very well in transferring motor power to the broth resulting in high mass transfer coefficient for oxygen consumption by the culture. But it appears that these also result in long mixing time that negatively affects some processes. Another option was to replace radial flow impellers with axial flow impellers to increase bulk mixing. Such impellers significantly reduce the time for fluid to mix between compartments caused by the impellers (Vrabel *et al*, 2000). Replacing the two top impellers with axial flow impellers was estimated to reduce the mixing time by 70%.

The third method of reducing mixing time was to add substrate not just at the top of the fermentor, but at additional points throughout the vessel. Vrabel *et al* (1998) developed a model suggesting that the addition of feed at one more feed location could reduce the mixing time by 75% and the addition of feed at three more feed locations could reduce the mixing time by 95%.

Modifications were made to a manufacturing fermentor by adding feed inlet locations. The process described above was improved significantly from about 50% of laboratory fermentor performance to about 90% of this same metric, as shown in Figure 3.

4. Conclusions

Pulse feeding experiments were carried out in laboratory fermentors. Assumptions of a mixing zone comprising 10% of the reactor volume and mixing time of 3 to 5 minutes gave results consistent with pilot plant and manufacturing fermentors, respectively.

These results indicate that substrate gradients brought about by insufficient mixing were primarily responsible for the reduced performance upon scale-up.

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