CHAPTER 4

MIXING IN FERMENTATION PROCESSES

James Y. Oldshue

Mixing Equipment Company
Rochester, New York

Mixing in the Fermentation Industry involves a large number of different kinds of operations. There are many kinds of laboratory mixing requirements, mixing involved in seed tanks, and the agitation involved in the typical large size fermentation tanks in the production plant.

In addition, there are many auxiliary tanks, some of which are involved in changing pH in the harvesting and recovery steps, and of course the many types of mixing operations that are carried out auxiliary to the main fermentation system.

The fermentations themselves have many different characteristics, from the traditional mycelial fermentation to the many viscous type processes, as well as the low viscosity bacterial type.

This review will relate to the general use of mixing in all these operations and lay down some of the basic principles involved that apply to a wide variety of fermentation characteristics.

PUMPING CAPACITY, FLUID SHEAR RATES, MACRO SCALE AND MICRO SCALE

All that the energy that is applied to a fluid mixer eventually appears as heat. The only way that energy can be converted to heat is through the mechanism of viscous shear. In viscous materials, this can happen uniformly through the entire mixing tank. In low viscosity, turbulent flow, large scale turbulent eddies transfer energy between smaller scale eddies. The transformation to heat takes place at the micro scale level where the scale is small enough for viscous shear effects to occur.

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Many standard references (1,2) describe in detail the mechanisms of flow and fluid shear but we will just summarize some of these concepts here, and show their application in later sections.

Fig. 1 shows a typical velocity from a radial flow impeller blade as measured by a high frequency response probe, such as a hot wire velocity meter, hot film velocity meter, or a laser doppler velocity meter. This is a general case where turbulence is present. The analysis is made by calculating the average velocity at a point as well as the fluctuating velocity component at that point.

By using the average velocity, and plotting velocity profiles at selected planes in the mixing tank, the shear rate, Fig. 2, is the velocity gradient, dv/dy and has the units of reciprocal seconds.

In viscous fluids, these shear rates operate throughout the tank at different magnitudes and scales. In turbulent flow however, the shear rate between the average velocities operates primarily on particles on the order of several hundred microns. The fluctuating velocity shear rates deal with micro scale processes that have particle sizes or cluster sizes of two hundred microns of less.

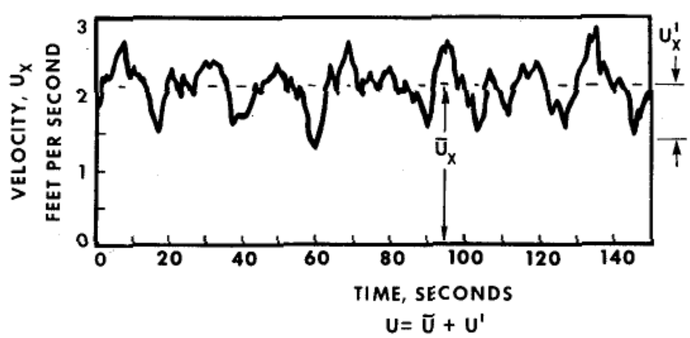
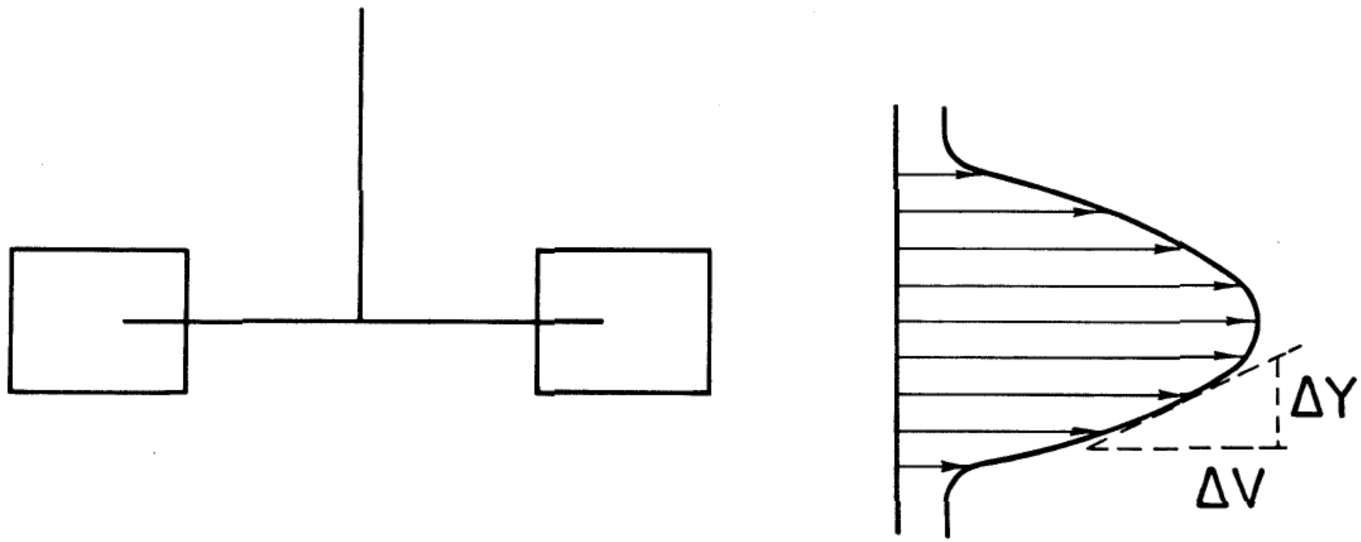


Figure 1. Typical velocity vs. time curve made by high frequency response velocity instrument in mixing tank.



$$\underline{\textbf{SHEAR RATE}} = \frac{\Delta V}{\Delta Y}$$

Figure 2. Velocity profile leaving the blade or radial flat blade turbine using average velocities at the measuring point to illustrate the calculation of shear rate.

Large-scale bubbles respond to the macro scale shear rates, and Fig. 3 shows how these shear rates can change on scale-up. There is typically a maximum shear rate around the impeller that increases with impeller tip speed, while there is also an average shear rate around the impeller that decreases with impeller speed. Fig. 3 shows that these two diverge on scale-up, tending to give a greater variety of shear rates in large tanks than in small tanks.

In addition, we can define an average in-tank shear rate, from a process standpoint, which is normally a factor of 10 or so less than the average shear rate around the impeller. In the remote parts of the tank there are also *minimum shear* rates that can be defined, which also decrease on scale-up.

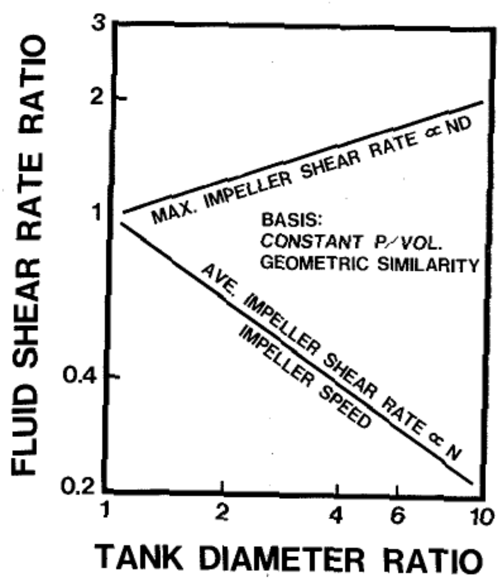


Figure 3. Typical illustration of increase in maximum impeller macroscale shear rate with scale-up and a decrease in the average impeller zone macroscale shear rate on scale-up.

Micro scale shear rates on the other hand are more related to the energy dissipation at a point, thus we would have to know more about the energy dissipation around the impeller and other parts of the tank to predict the distribution of micro scale shear rates.

It turns out that macro scale shear rates are highly sensitive to scale and geometry, and the use of non-geometric scale-up and scale-down techniques, as reported in other references (7) are powerful tools to control selected parameters in the macro scale shear rate range. On the other hand, micro scale relationships, which are often involved in chemical reactions, are more sensitive to energy input, and are not as sensitive to scale and geometry of the equipment.

In all cases, it must be recognized that shear stress is the product of viscosity and shear rate, shear stress = μ (Shear Rate), thus at any shear rate, the shear stress produced is directly proportional to the viscosity at that shear rate.

Within any given tank size, we have the ability to change the pumping capacity of the impeller, Q, and the average shear rate, (AIZSR), around the impeller by the relationship, $Q/AIZSR \propto D^{8/3}$, where Q is impeller pumping capacity, AIZSR is the average impeller-zone shear rate, and D is the impeller diameter.

SOME RECENT WORK ON VELOCITIES

A laser doppler velocity meter is of very great value in measuring the velocities and energy dissipation in transparent liquids. Fig. 4, shows the schematic of the laser doppler equipment, while Figs. 5, 6, 7, 8, and 9 show the typical traces of flow patterns studied by means of a laser doppler velocity meter.

With the ease of measuring these velocity profiles, better characterization of impeller flow pattern has been possible, and impellers with high pumping capacity and consequently low shear rates have been developed, to extend that end of the impeller spectrum.

Fig. 5 shows an axial pitched-blade turbine 2D off bottom illustrating the radial component. Fig. 6 shows an airfoil type axial flow impeller at the same 2D elevation showing greater axial flow for that impeller, with almost zero radial component.

Figs. 7 and 8 show the same impellers about 1D off bottom.

Fig. 9 shows a radial flow impeller with blades below a full disc covering the top of the blades.

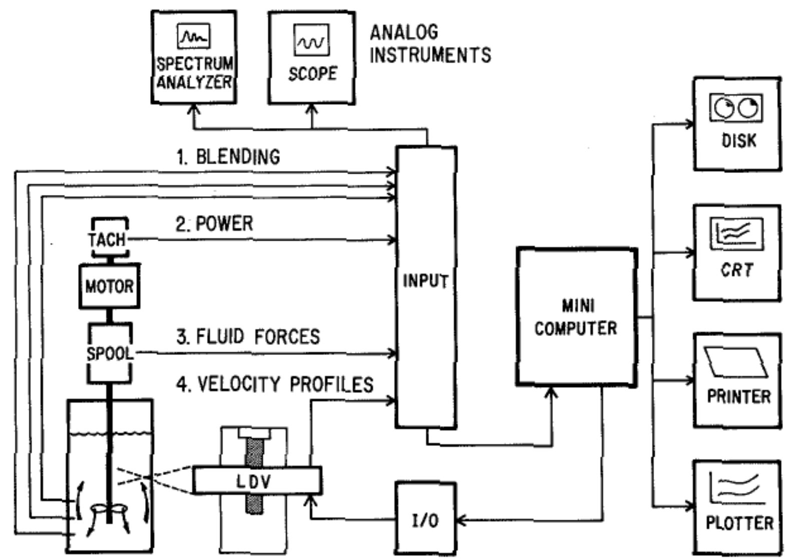


FIGURE 5. INTEGRATED LABORATORY.

Figure 4. Schematic of laser doppler velocity measuring equipment.

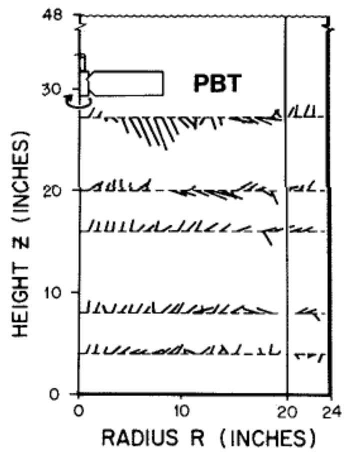


Figure 5. Typical velocity scan produced by laser equipment for pitched blade turbine mounted two impeller diameters above the tank bottom.

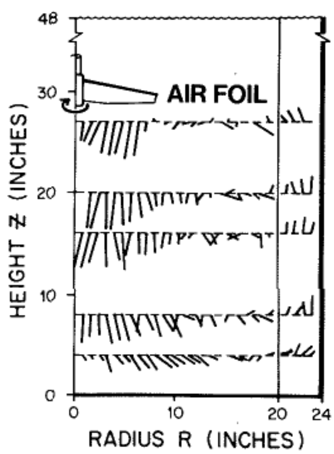


Figure 6. Typical laser scan velocity profile of air foil axial flow impeller two diameters above tank bottom.

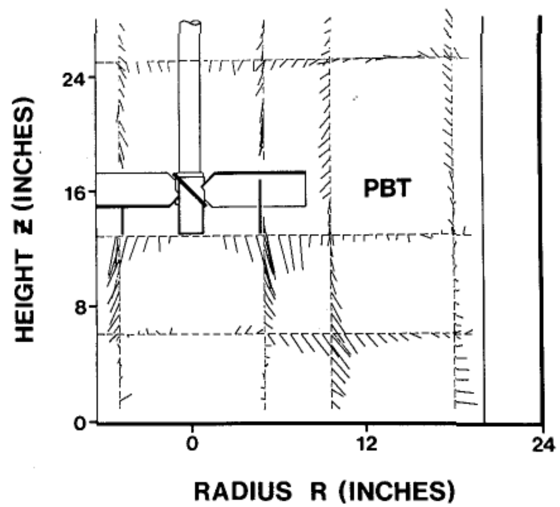


Figure 7. Typical laser scan for pitched blade turbine mounted one impeller diameter above tank bottom.

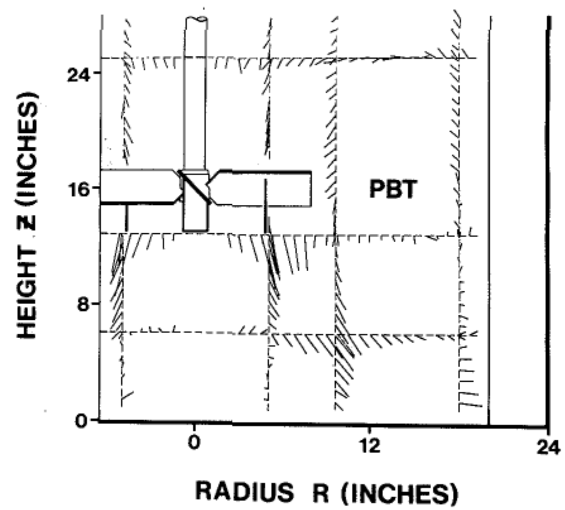


Figure 8. Typical laser scan velocity profile for air foil axial flow impeller located one diameter above tank bottom.

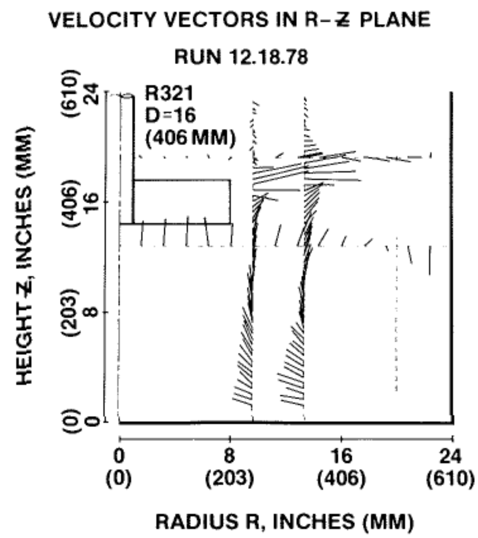


Figure 9. Typical velocity profile from laser doppler velocity meter for radial flow impeller having full diameter disc on top and radial blade extending beneath the disc.

GAS-LIQUID MASS TRANSFER FERMENTATION STEP

The traditional flat blade turbine impeller operated on a central mixing shaft with gas introduced at the bottom of the tank is still the mainstay of most production-scale fermenters and many pilot plant systems. These impellers provide good mass transfer characteristics throughout the fermentation batch. They are essentially high shear rate, low pumping capacity impellers which are generally indicated for mass transfer applications. However, the D/T ratio in many fermenters is around 0.35 to 0.45, which increases the pumping capacity vs. the shear rate of these impellers over what would be optimum for purely gas-liquid mass transfer alone.

Typical curves correlating the mass transfer coefficient, K_Ga, which is used for scale-up rather than mass transfer rate, are given in Fig. 10. In order to calculate the mass transfer coefficient, a suitable gas-liquid mass transfer driving force is needed. Fig. 11 shows a typical pilot fermenter. At Z/T = 1, (where Z = batch depth and T = tank diameter) and at high power levels, it is possible to have a well-mixed gas phase in which the exit gas composition is suitable to use for calculating the mass transfer driving force.

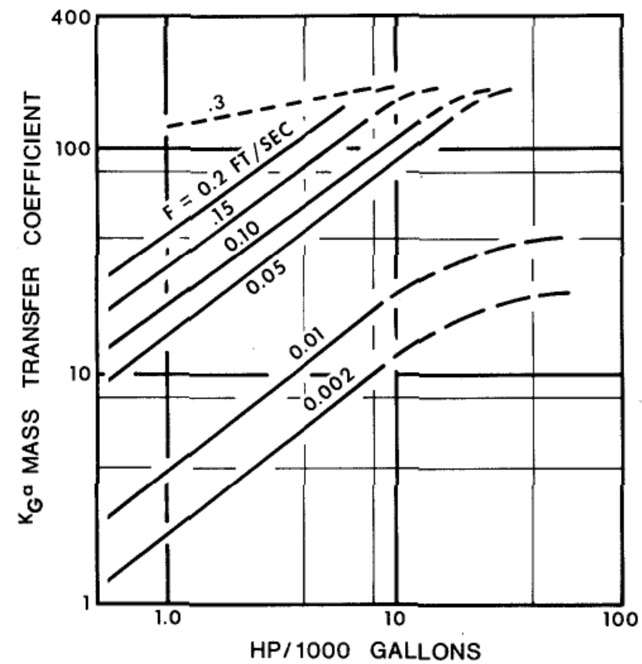


Figure 10. Typical correlation of mass transfer coefficient, K_Ga, vs. impeller power and constant linear superficial velocity.

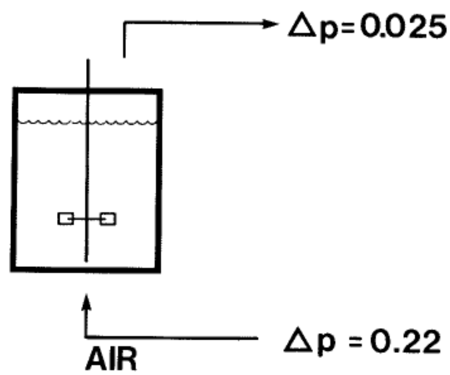


Figure 11. Schematic of gas-liquid mass transfer driving force for pilot scale tank.

In all cases, a P* value is needed, which is the equilibrium partial pressure corresponding to the dissolved oxygen level in the tank. Depending upon the fermentation, this may or may not be readily available. If it is not available, then P* must be estimated in terms of percentage of saturation. Even if there is some error in this estimate, if it is used both in the pilot plant and the full scale calculation, the error involved is largely negated by being used in calculations.

A large fermenter, Fig. 12, shows that it is very unlikely that the gas phase will be mixed from top to bottom. It has been my experience that a log mean driving force between the bottom and the top of the tank is most suitable.

There has been much interest in the use of air agitation only; either in terms of sparging air into the tank and with no internal fittings, or in using a draft tube arrangement and introducing the air in the draft tube area.

The general comparison which was presented in a summary paper by Robinson and Moo-Young (3) indicates that at any gas rate, adding a mixing impeller to the system increases the mass transfer rate over that for using air alone. When high mass transfer rates per unit volume of the fermenter are required, an impeller and gas system is more suitable than air alone. However, for medium or low uptake rates, it is possible to use air sparged fermenters, and there are

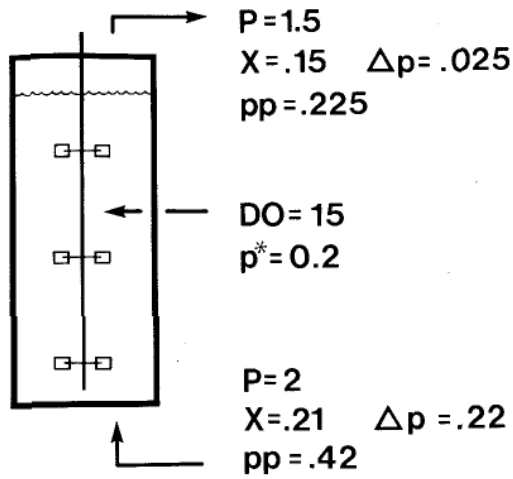


Figure 12. Typical gas-liquid concentration driving force measurements for large scale plant fermenter.

some examples of those in operation around the world. However, air mixing is not particularly conducive to effective blending, solid suspension and/or heat transfer, so that using impeller-type systems is necessary most of the time.

In a typical batch fermentation, Fig. 13, viscosity increases with time, and several investigators (4) have shown that there is a reduction in mass transfer coefficient, K_Ga, with increase in viscosity as the run progresses.

Fig. 14 illustrates the situation that can happen on fermentation with two different full-scale mixers. Most industrial fermenters today are still single-speed. Let's assume that in Fig. 14, Mixer B is installed. It has a high mass transfer rate at the beginning of the run, but as the viscosity increases, the mass transfer ability of the mixer is decreased where it no longer can meet the uptake rate of the organism. Productivity of the fermenter will be less than maximum since the required process of mass transfer during the later stages is not available.

Another possibility is to install a larger mixer, Mixer A, Fig. 14, which will have the mass transferability at the higher viscosity of the fermentation. It will meet the full mass transfer uptake requirement of the organism and allow full productivity to be established.

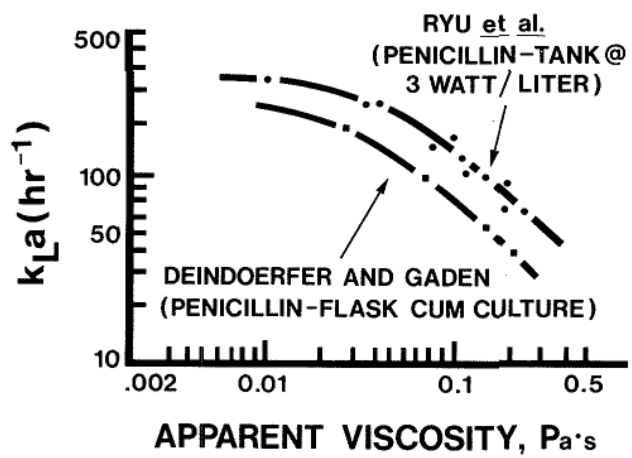


Figure 13. Data from Ryu, et al., on decrease in K_Ga with increase in viscosity of fermentation broth.

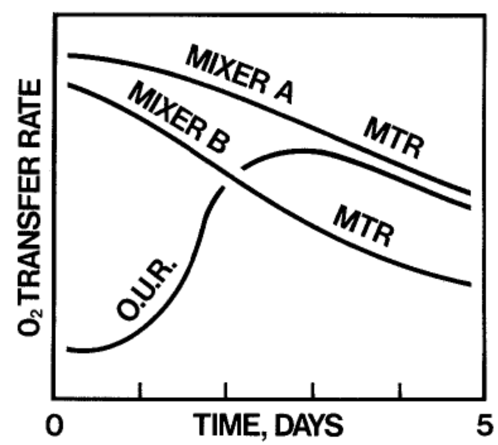


Figure 14. Oxygen mass transfer rate for two difference mixers, A and B. Mixer A satisfies oxygen uptake requirement during the entire fermentation cycle. Mixer B satisfies it initially, but is deficient in latter part of fermentation cycle.

This is further illustrated in Fig. 15 by Mixer I. However, it has such a high mass transfer ability during the first few days of fermentation, that the organisms may be overstimulated. There is some concern that this may cause an initial rapid increase in yield, or may result in lower total yield, Fig. 15 (Yield I), compared to Mixer II. Mixer II has a two-speed motor or a dual power number impeller such that the power can be reduced during the first part of the fermentation. This will maintain the mass transfer rate at just what is needed, and will not overstimulate the organisms with high dissolved oxygen levels during this first period. This may give a higher ultimate yield, Yield II, Fig. 15. There is some indication that the ability to continuously monitor dissolved oxygen level and adjust the mixer speed accordingly (with a complete, variable speed drive) will result in maximizing yield for fermentation. It would also be helpful in minimizing horsepower input. Pilot plant equipment is usually equipped with this kind of monitoring equipment and variable speed capability, but this has not yet been used on any significant number of full-scale fermenters.

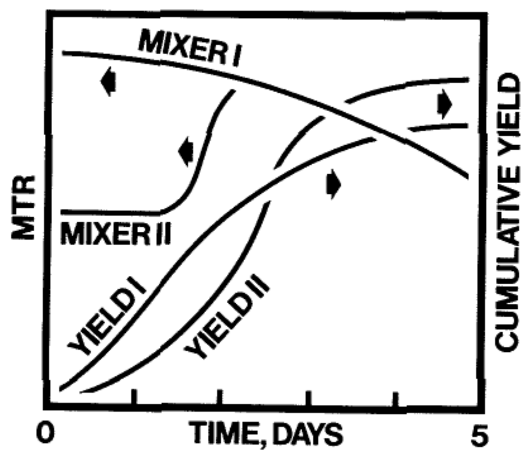


Figure 15. The constant speed mixer, Mixer I, which satisfies the oxygen requirement during the entire fermentation cycle, but may overstimulate organism to give yield I curve. Also illustrated is Mixer II, which is a 2-speed unit, which reduces oxygen mass transfer in the first part of the cycle, but is sufficient for requirement in the more viscous, latter portion of the cycle, giving yield II curve.

SCALE-UP

Scale-up is a primary concern of fermentation investigators. In order to understand the characteristics of scale-up relationships, a few general remarks are in order about the fluid mixing capability and characteristics of small and large tanks. Quite typically, a large tank will have a longer blend time, a higher maximum macro scale shear rate around the impeller, a lower average macro scale shear rate around the impeller, and a lower average tank shear rate. In addition, if equal volumes of air per unit volume of liquid are used, the large tank will have a higher linear superficial gas velocity, which is obtained by dividing the air flow rate by the cross-sectional area of the empty tank. This higher superficial gas velocity will raise the power per unit volume due to air expansion in the tank. This will tend to give more of an air-controlled flow pattern in full scale rather than a mixer-controlled flow pattern.

These high superficial velocities will have an effect on the power drawn by the impeller, and on foaming characteristics in the tank, and will actually tend to increase the blend time over what it was in the pilot tank. This means that the large-scale fermenter mixing parameters are from the small side and some people wonder why they perform as similarly to small scale equipment as they usually do.

Assuming that the pilot plant unit has been run with a conventional flat blade disc impeller for which the blade width is 1/5 of the impeller diameter and the blade length is 1/4 of the impeller diameter, drastic changes in impeller or system geometry must be made to a full scale unit if pumping capacity is to be increased and shear rates are to be decreased. This may require the use of more impellers, their blade width ratios may be increased from 1/5D to possibly 1/4D or 1/3D, and the D/T ratio increased in the full-scale plant.

All of these changes will probably still not produce the same blend time, turnover time or shear rates in the full-scale fermenter. This means that estimates must be made for the length of time a cluster of fermentation cells is away from a high level mass transfer zone, particularly if the tank is equipped with baffles and helical transfer coils. This is especially important when particles reside in remote parts of the tank, away from the main mass transfer field.

It is difficult to use a standard geometry in the pilot plant and be able to change that geometry sufficiently in the full scale to maintain most of the mixing parameters in the desired range. Therefore, we must think seriously about using a modified geometry in the pilot plant so that scale-up to more conventional type impeller combinations will be possible in full scale.

This means that the impeller blade height should be reduced to probably 1/15D or 1/20D, with the important correlary requirement that the blade height shall never be less than two or three times the diameter of the largest gas bubble that is of interest in the mass transfer step.

It also means that the D/T ratio in the pilot plant ought to be more like 0.2 or 0.25 so that a more typical 0.35 to 0.45 D/T can be used in the plant with effectiveness.

A typical pilot plant run gives a plot of K_Ga vs. gas rate with a lower rate and power level in the pilot plant as shown in Fig. 16. The box on the right-hand side of the figure shows the typical pilot plant area, and since the superficial gas velocity is increased in the plant scale-up, it is possible to move plant unit design to the left box of the figure. This indicates a much lower power per unit

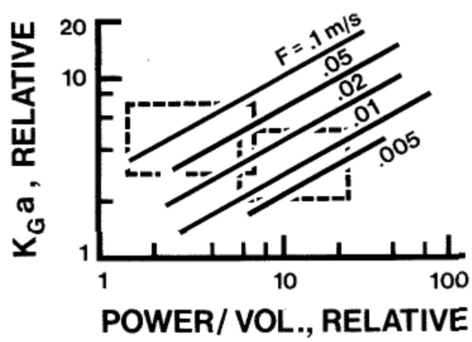


Figure 16. Typical data of K_Ga vs. power and constant gas rate parameters illustrating typical pilot plant area at right and full scale design area at left.

volume is possible. However, the blend time will be increased nevertheless in the scale-up. Since the liquid-solid mass transfer rate is affected by the mixer power level, it is not usually possible to reduce the power level very much from what it was in the pilot plant, even though the plant unit has a much higher linear superficial gas velocity.

The net effect is that mass transfer ability of a large size fermenter is usually more favorable to the process than it was in the pilot plant.

The geometry of the full-scale plant tank should be considered from many different aspects. The traditional, tall, thin tank came from the fact that there is a maximum diameter allowed for tanks to be transmitted over highways. So to obtain the higher volumes required for large scale production, the tanks had to be tall and relatively small diameter. This results in a good combination from many aspects of gas-liquid mass transfer. However, it does have the effect of giving increased superficial gas velocities over that which a lower profile geometry, with a Z/T ratio of 1 or 2, would yield.

Actually, various lower profile tanks with a Z/T ratio of 1 to 1.5 can be considered, and the use of horizontal cylindrical tanks can also be part of the geometry study. With the right type of mass transfer data from the pilot plant, a correlation will be available so that full-scale design can be developed for a wide variety of tank shapes. Thus, an overall evaluation can be made.

CARBON DIOXIDE DESORPTION

Desorption of CO_2 is thought to be an important part of the fermentation process. However, many of the things that improve gas absorption, such as increased liquid depth and head pressure in the tank, inhibit the desorption of CO_2.

CO_2 desorption should be measured on both the pilot scale and full scale runs. Fig. 17 shows a typical result.

BLENDING IN FULL SCALE

It is possible to estimate the mass transfer coefficient at various places within the mixing tank. Obviously, they are much higher near the impeller zone than in the rest of the tank. The uptake rate of the organism can be used to estimate the reduction in DO around the particle based on its circulation time in the system. If these calculations show that an appreciable fraction of the particles are deficient in dissolved oxygen, it may be that irreversible damage has occurred, and which may be one cause for yields not being as high as they should be.

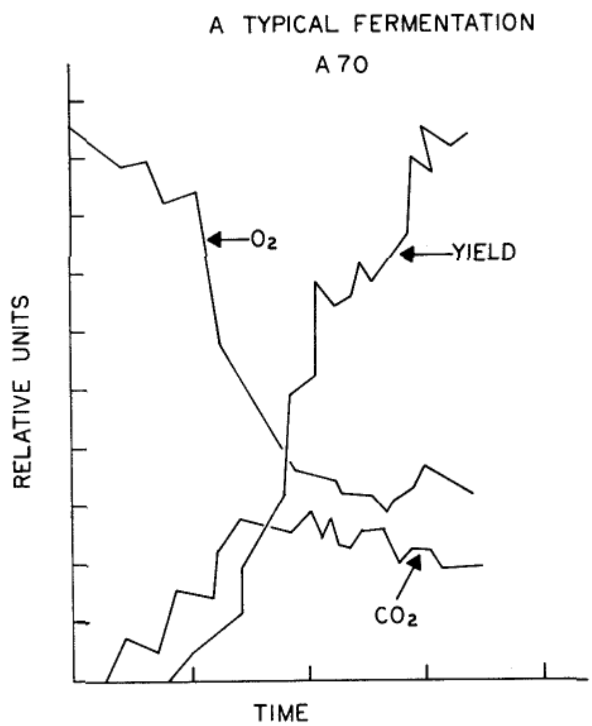


Figure 17. Typical measurements of dissolved oxygen, dissolved CO_2, and fermentation yield from pilot or production size fermenter.

Circulation time in large tanks has been shown by Middleton (8) to be much longer than would be calculated by the ratio of pumping capacity to the volume of the tank. In fact, the particles do not follow a general overall flow pattern in the tank but assume smaller circulation patterns within the main pattern. The flow pattern in a 1,000 gallon tank with a Z/T = 1.0 and a single impeller can be equivalent to 2 to 10 tanks in series as far as the particle velocity in the system is observed.

The presence of gas bubbles also increases the blend time in an homogeneous liquid. An increase in superficial gas velocity similarly increases the blend time.

MIXER COSTS

As the viscosity of the fermentation broth increases, the mols of oxygen that can be transferred for a given power level decreases. For example, to give a relative viewpoint, at 10/grams/liter of solids, let's say that the ability of the mixer to transfer oxygen will be ten mols of oxygen per megajoule. At 20/grams/liter solid, the mixer can transfer 6.4 mols of oxygen per megajoule and at a concentration of 40/grams/liter, the mixer can transfer 4 mols of oxygen per megajoule. The productivity of a fermenter is increased dramatically with the increased cell concentration, but it is true only at a higher power cost. However, the net total cost of production will be less with the higher cell concentration (5).

CHEMICAL REACTIONS

The micro scale velocity fluctuations shown in Fig. 1 can be calculated as a Root Mean Square, RMS, value and plotted as in Fig. 18. The ratio of fluctuations to average velocity are shown in Fig. 19. Around the impeller the fluctuations are about 50%. This means that a reagent added to a reactive solution in the tank at the impeller will experience an instantaneous concentration change of plus or minus 50%. If the reaction speed was such that it could detect this concentration fluctuation, there would definitely be a difference in the reaction rate. If there were a series of competitive, consecutive reactions, there might well be a difference in the selectivity of the products produced by that sort of situation. Bourne (6) has been studying the principles involved when mixers make a difference in the selectivity of chemical reaction, and further reference to

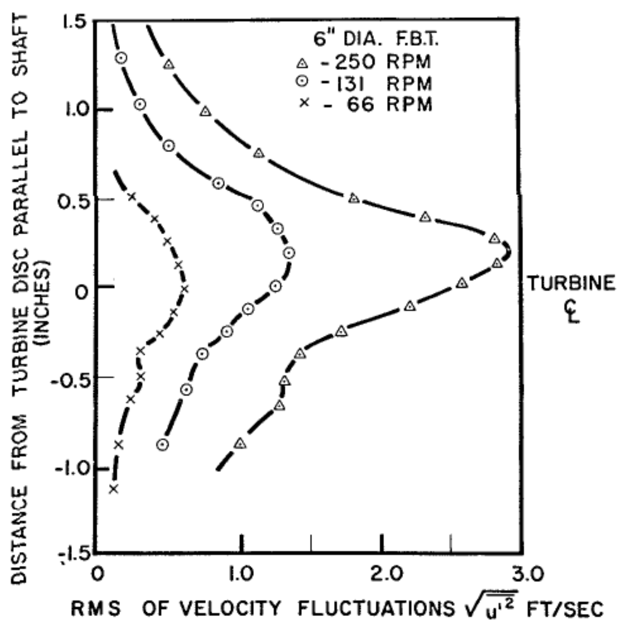


Figure 18. Plot of the Root Mean Square of velocity fluctuations in turbulent fluid at three different impeller speeds at various distances above and below the impeller center line for radial flat blade disc turbine.

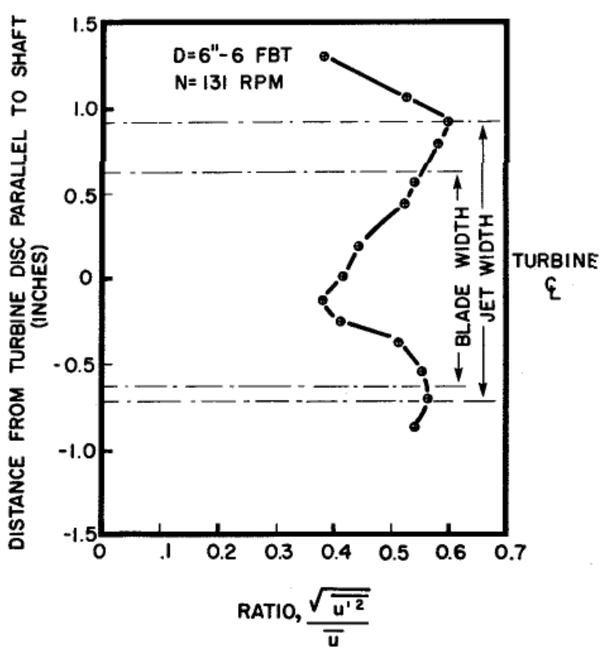


Figure 19. The ratio of the Root Mean Square velocity fluctuation divided by the average velocity at a point for a radial flow flat balde turbine.

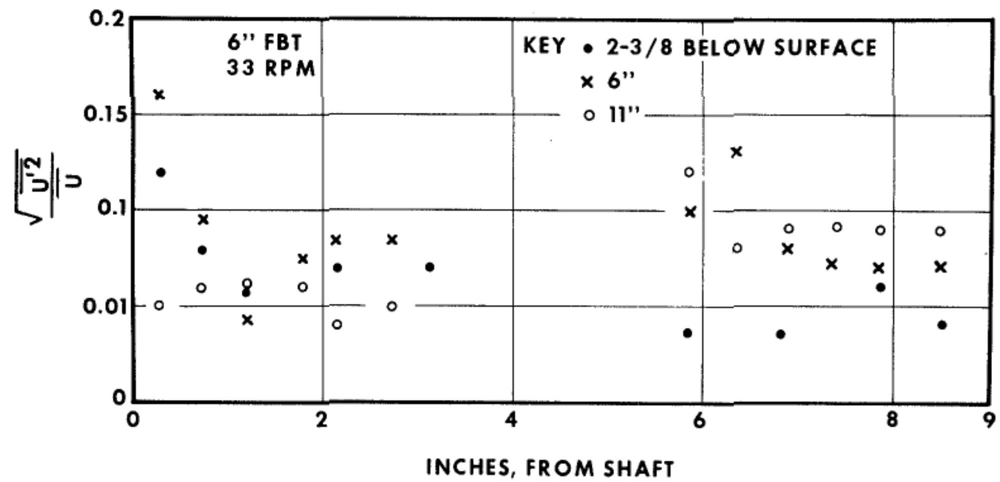


Figure 20. Ratio of Root Mean Square velocity fluctuation to the average velocity of the point for various distances from the shaft at various depths in the tank.

his work is recommended. Paul (9) also showed that the Root Mean Square, RMS, value at the point of introduction did correlate with the observed selectivity of a competitive, consecutive reaction system.

In the more remote parts of the batch, near the surface, the RMS value is only 5% to 10% of the mean, Fig. 20. Thus, for chemical reaction, experiments on the effects of feed introduction -points and other variables are important. Tests should also be conducted to determine the best way to introduce the gas, i.e., by open pipe, multiple pipes or sparge rings. The results of these experiments will provide a good indication of the best methods to be used for a given process.

NOMENCLATURE

N	Impeller speed
D	Impeller diameter
Z	Liquid level
T	Tank diameter
$K_G a$	Gas-liquid mass transfer coefficient, based on gas phase
$K_L a$	Gas-liquid mass transfer coefficient, based on liquid phase
D.O.	Dissolved oxygen
$\frac{dv}{dy}$	*Shear rate*
RMS	Root Mean Square
Q	Impeller pumping capacity
H	Impeller velocity head
OUR	Oxygen Uptake Rate
MJ	Megajoule

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