MIXING IN THE MECHANICALLY AGITATED BIOREACTOR: 'A MATTER OF BEING STIRRED, NOT SHAKEN'

Shaliza binti Ibrahim
Department of Civil Engineering, University of Malaya, 50603 Universiti Malaya, Kuala Lumpur
E-mail: shaliza@um.edu.my

ABSTRACT

Stirred vessel bioreactors are often equipped with the standard 6-blade disc turbine without much thought for process specifications. Variations in the system parameters can in fact give rise to significant differences in mixing requirement, and failure to operate at the right conditions can result in undesirable outcomes. Biological systems are sensitive to bioreactor hydrodynamics since it directly affects transport processes at the molecular level. In addition, extracellular products and filamentous microbes contribute to drastic changes in the broth characteristics over the course of fermentation, that would in turn alter the mixing needs. Poor mixing or over mixing can both have adverse effects on product quality, but inefficient equipment design leading to excessive energy requirement often goes unnoticed if there are no apparent effects on the product. This paper summarizes the basic considerations to be made for given mixing duties so as to enable operations in a calculated rather than random manner.

Keywords: *Impeller, Mixing Power, Multiphase Mixing and Rheological Effects, Stirred Vessel*

1. INTRODUCTION

The advent of modern biotechnology has brought a new meaning to the term fermentation, to include aerobic cultures using isolated microbial and non-microbial cells or enzymes, along with the traditional anaerobic microbial processes [1]. Subsequently, the bioreactor has evolved from a simple wooden keg into a sophisticated rig capable of running aseptic processes with automated control and monitoring for continuous or intermittent supply of air, substrate and additives [1]. In accommodating a culture to produce the desired outcome, the bioreactor can have various configurations, but central to the design of a bioreactor is the mixing aspect. In bubble column and air lift bioreactors mixing takes place through the circulation of sparged air bubbles, which limits these reactors to low and moderate viscosity systems. The stirred tank reactor (STR) has a mechanical agitator to mix its contents and this method is favoured for its versatility in handling a wide range of fluid properties. Despite its importance, mixing is often overlooked as an easy step to create chaotic motion, attainable with simple standard equipment. But the lack of systematic consideration of relevant mixing parameters can affect productivity and product quality, or incur high operating costs due to over-design [2].

The mixing task is usually looked at from the perspective of the phases (liquid, solid, gas) involved, and a fermentation broth contains multiple phases with the cells as solid particles, and gas in the form of air bubbles, coexisting in an aqueous liquid medium. When a non-aqueous liquid is added such as oleic acid substrate in the Pseudomonas culture for polyhydroxyalkanoate (PHA) production [3], a fourth phase is involved, usually at a very low percentage that nevertheless needs to be made accessible to every cell. In all cases the aqueous medium acts as a continuous matrix into which the gas is dispersed as bubbles, the solid particles fully suspended, and the non-aqueous liquid phase dispersed as droplets. Dispersed bubbles, and suspended particles and droplets maximise the surface areas of contact between the respective phases, thus supporting the inter phase transfer of nutrients and products. Meanwhile, a good turn over of the bulk fluid allows for an even distribution of heat and momentum to eliminate hot spots and dead zones.

The challenge is that there is yet to be a single impeller that can optimally carry out all the required functions simultaneously. This paper summarily presents the effects of parameters, which outline the criteria for choosing the right type of mixer for a particular operation.

2. THE STIRRED TANK

Figure 1 is a schematic of a vertical, cylindrical stirred vessel of diameter T(m) with a base that can be flat, conical, dished or contoured. Liquid of density, ρ(kg/m^3) and viscosity, (Pa.s) is filled to a level H(m). An impeller of diameter D(m) is mounted on a central top shaft at a clearance of C(m) from the base. Off-centred and bottom shafts are sometimes used too. N(rps) denotes the rotational speed, whilst DN(m/s) is the impeller tip speed. Q(m^3/s) is the impeller volumetric pumping rate. Air is conventionally sparged through a pipe or ring sparger, and the gas flowrate is denoted by Q_G (m^3/s). Solid particles if present are represented by their concentration, X(%), size, d_p(m), relative density, ρ_P and shape.

The impeller rotation gives rise to torque, τ(N) and power, P(W) = $2\pi N\tau$. P is the power actually transmitted into the fluid

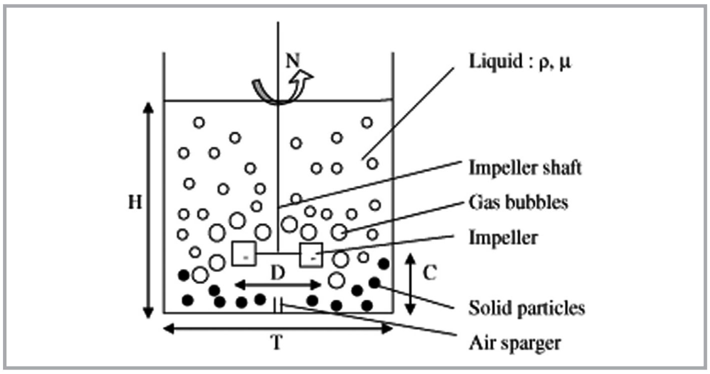

Figure 1: Three-phase mixing in a flat-based stirred vessel

after friction losses in the shaft bearing, not the power required by the motor. Methods devised to measure this power include the use of strain gauges coupled with a telemetry system [4], and suspending the tank on an air bearing to detect the torque [5]. Measurements taken during aeration give values for gassed power, P_G.

The mean specific energy dissipation rate is given by $\Sigma_T = P/\rho V_L$(W/kg) where V_L(m^3) is total liquid volume. Typical values of Σ_T range from about 1.0 W/kg to 5.0 W/kg for low and high viscosity fermentations, respectively.

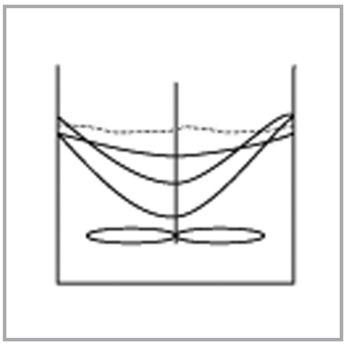

Figure 2: Gross vortexing

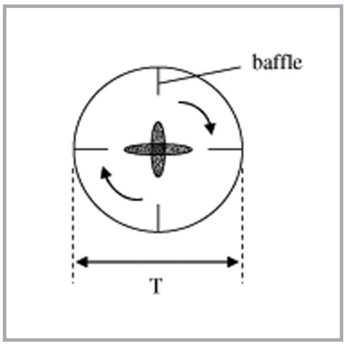

Figure 3: Top view of a baffled vessel

3. DIMENSIONLESS GROUPS

The stirred vessel variables can be grouped as:-

1. Reynolds number, $Re = \rho ND^2/\mu$, which represents the ratio of inertia to viscous forces. Only the liquid properties are used to calculate Re. In the stirred vessel, the laminar regime occurs at Re<10, transitional regime in the range $10 \leq Re \leq 10^4$, and turbulence at $Re \geq 10^4$.
2. Power number, $Po = P/(\rho N^3D^5)$ and gassed power number, $Po_G = P_G/(\rho N^3D^5)$
3. The agitator flow number, $Fl = Q/(ND^3)$ is constant for a given impeller geometry.
4. $Fl_G = Q_G/(ND^3)$ is the gas flow number.
5. Froude number, $Fr = N^2D/g$, the ratio of inertial to buoyancy forces, accounts for the formation of a central vortex due to swirling (Figure 2) at Re > 300. The vortex deepens as the speed increases, causing instability, ingestion of air, and poor top to bottom mixing. It is eliminated by using baffles to break the swirling effect. The standard is to use four baffles, each with a width 1/10 of the tank diameter (Figure 3). In multiphase systems, Fr may be considered for the difference in density between two phases.
6. Geometric terms are the ratios between the various dimensions in the bioreactor, such as impeller-to-tank diameter, D/T, clearance-to-tank diameter, C/T, blade thickness-to-tank diameter, x/T, liquid height-to-tank diameter, H/T (also known as aspect ratio), etc. Systems with identical geometric ratios are *geometrically similar*.

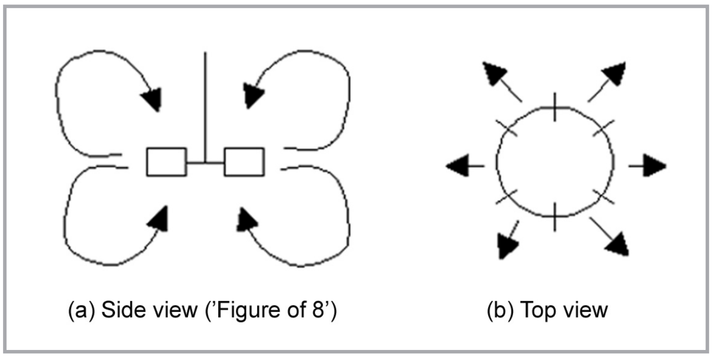

Figure 4:** **Bulk flow from a radial impeller

4. SINGLE –PHASE LIQUID MIXING

4.1 BASIC FLOW PATTERNS

(a) Radial 'High Shear/Low Flow' Impellers

The classic 6-blade disc turbine (6DT) affectionately known as the Rushton turbine is a typical radial impeller which pumps fluid outward from the centre, creating a 'Figure of 8' profile as shown in Figure 4. The flow pattern is maintained over the whole range of Re except at very low clearances of about T/6 where the flow has been reported to dip to the vessel corner instead of going radially to the wall [6].

The radial impeller has each of its blades' full vertical width shearing on the fluid as the impeller rotates in a circular tank (top view in Figure 5), attributing to a high shearing action, and this is what makes the Rushton turbine so efficient as a gas disperser. (More on this will be explained in Section 5)

(b) Axial 'High Flow/Low Shear' Impellers

Axial impellers have inclined blades; about 30 from the horizontal in the case of the 3-blade marine propeller, Lightnin' A310 and Chemineer HE-3, all of which produce high axial flow

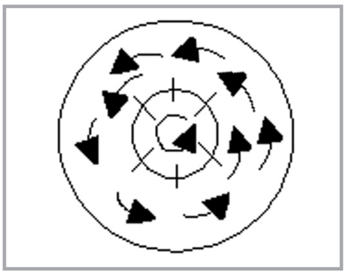

Figure 5: Shearing direction

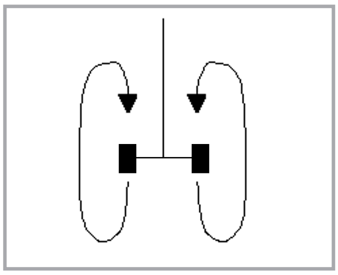

Figure 6: Axial downward flow

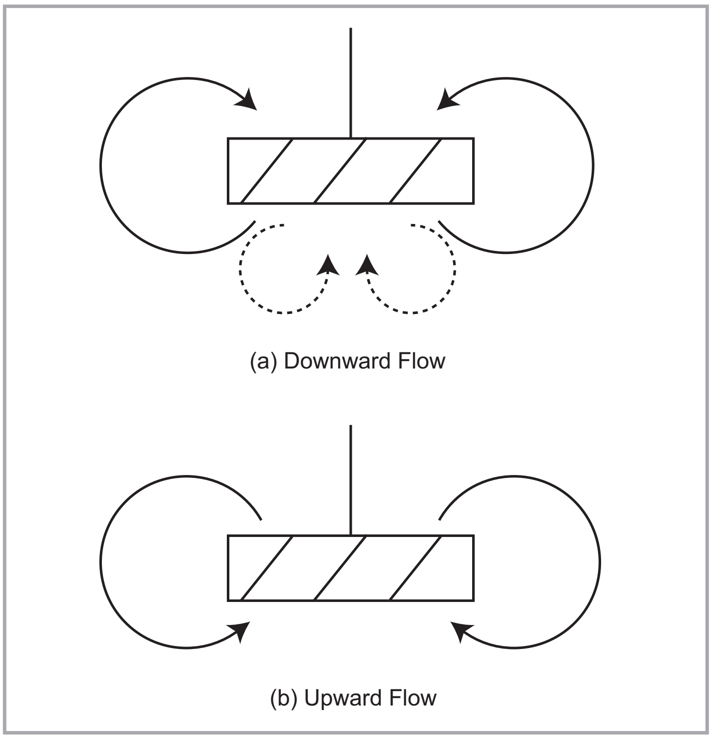

Figure 7:** **Flow from mixed flow impeller

(Figure 6) pumping down, for excellent top to bottom mixing [6].

There are 'mixed-flow' impellers having flat blades inclined at 45°-60°. These impellers produce radial and axial components in the flow. They can pump down or up as shown in Figures 7 (a) and (b).

Large impellers designed for high viscosity systems such as the helical ribbon or Ekato InterMIGs also produce axial type flow. The InterMIG comprises two up-pumping inner blades connected to four smaller down-pumping vanes. Used in pairs mounted perpendicularly from each other, they produce chaotic motion in the turbulent regime.

Streamlined flow at low Re impedes the axial components of flow and the direction becomes more radial, so much so that at Re<200 a pattern akin to the 'Figure of 8' occurs for the 3-bladed HE-3 and InterMIG impellers [6].

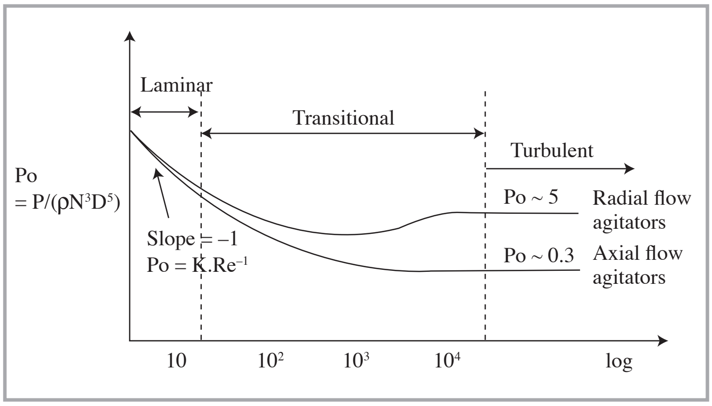

Figure 8: "Power curves" (single phase liquid mixing)

4.2 POWER CORRELATION

Log-log plots of Po versus Re (Figure 8) in single phase liquid mixing can be used to predict mixing power for a given condition. Reference to the plots stipulates that the systems have geometric similarity including standard baffles. Variations in geometric terms as well as scale will shift the power values although the characteristic shape of the curves is retained [6].

Regardless of impeller type, the curve is a straight line with a slope of -1 in the laminar regime, and has a constant Po value in the turbulent regime. The turbulent Po values are frequently referred to for impeller specification. The radial impeller Po of about 5 in the turbulent regime is considered high and is attributed to its high shearing action; while the axial impellers turbulent Po average around 0.3 to 0.6.

In the transitional regime the radial impeller curve dips slightly before rising to reach the plateau while the axial impeller shows a steady drop in Po as Re increases. Hence, for systems where viscosity increases over time, as for filamentous microbes and extracellular viscous products such as xanthan gum, the motor must be able to handle the increase in power demand with the drop in Re due to viscosity increase if an axial type impeller is used.

5. DISPERSION OF GAS IN LIQUID
5.1 IMPELLER HYDRODYNAMICS – THE FUNDAMENTALS OF GAS DISPERSION

The 6DT is usually chosen for the dispersion of gas in liquid at low viscosity. Rotating in liquid only, flow around its disc and blade edges form a pair of trailing vortices on the back of each blade [7], and the high spiralling velocity creates a low pressure region into which sparged air is immediately attracted before breaking away as

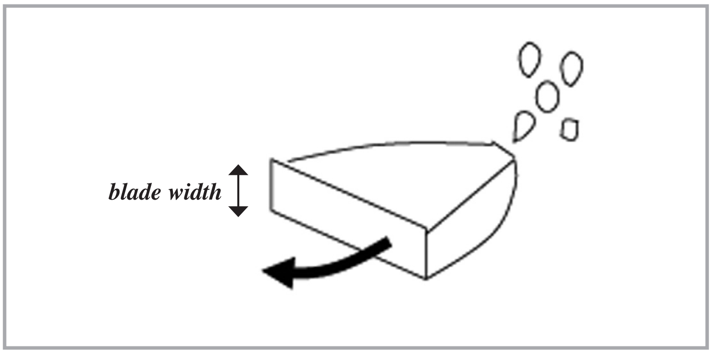

Figure 9: A large cavity behind an impeller blade

bubbles into the bulk fluid. Small amount of tiny bubbles moving through the vortices without changing the vortex structure form what are known as *vortex cavities* [7]. Increasing the gas flowrate causes bubbles to coalesce and partly attach to the blade, to form *clinging cavities*. With more gas, three clinging cavities on alternate blades become *large cavities*, which are single blobs of bubbles occupying the back of the blade. Eventually all six blades of the turbine have large cavities.

The discovery of ventilated cavities was profound in explaining the mechanisms of bubble dispersion in the stirred vessel. Equally exciting had been the observation that mixing power drops [8] by 10-20% when vortex and clinging cavities are formed, and then to a dramatic 40% of ungassed power when large cavities are formed. The drop is explained by increased pressure behind the blades, which reduces the drag in rotation.

Although less power means lower operating cost, the loss of energy imparted into the medium does not benefit oxygen transfer to cells since the mass transfer coefficient, k_La is directly proportional to P/V [9]. Ways to reduce the drop in power during aeration include using a ring sparger of diameter similar to the impeller diameter, increasing the number of impeller blades from 6 to 8 or twelve (odd number of blades has been observed to cause instability), or to use multiple impellers. An alternative design is a radial impeller with the blades curved to the back such as the Scaba SRGT [10]. The concave blades restrict the formation of large cavities that causes a large power drop. Work with 6-curved blade (6CB) impellers have shown that there is less drop in power under gassed conditions compared to using the 6DT, as illustrated in Figure 10 [11].

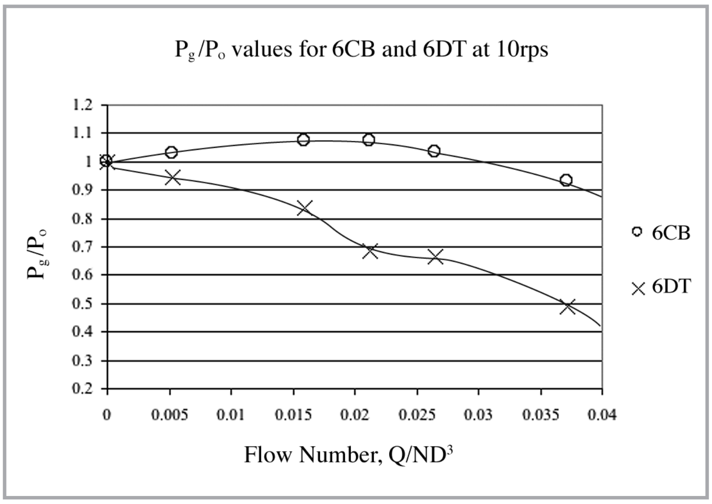

Figure 10: Reduced power drop with a curved blade impeller

5.2 GAS-LIQUID BULK FLOW

Gas dispersion with the 6DT undergoes a transition from *flooding* which occurs when the agitator speed is too low that it is overwhelmed by bubbles, to *loading* when the speed is increased and bubbles start to move below the impeller after occupying the region above the impeller. With adequate impeller speed, *complete dispersion* occurs and bubbles are well dispersed above and below the impeller. This is the desired operating condition for gas-liquid mixing in a stirred vessel. Complete dispersion usually coincides with clinging and large cavities. Correlations are available to determine the minimum speed to prevent flooding, N_F [12] and to achieve complete dispersion, N_{CD} [2].

Axial and mixed-flow impellers pumping down in gas-liquid systems are prone to flooding, asymmetric dispersion and power instability. These are partly attributed to the impeller pumping direction that opposes the flow of bubbles. Up-pumping mixed flow tend to perform better under gassed condition. The instability problems with down pumping impellers are exhibited even by novel hydrofoil impellers, namely the Lightnin A315 and Prochem Maxflo T, which have been developed for improved gassing and higher pumping capacity, while vibrations have been reported for InterMIGs [10]. The APV-B2 [10] pumping up or down is a more recent hydrofoil designed to suppress torque fluctuations and effectively disperse gas with negligible power reduction, as the contoured blades limits cavity formation.

6 SOLID SUSPENSION IN LIQUID

Down pumping axial and 6-bladed mixed-flow impellers are most efficient for suspending particles from the bottom, with $0.4 \geq D/T \geq 0.35$ being optimal for Re > 200. A contoured tank base is better to eliminate particles settling in the centre and the side [13]. For Re<200 a single InterMIG and larger D/T of the axial impellers are more efficient [14]. A commonly used correlation to estimate the minimum speed to achieve just-suspension, N_{js}, is the empirical correlation by Zwietering [15] based on the criterion that no particles remain stagnant for more than 1 or 2 seconds.

Since biological cells are almost neutrally buoyant in water, their suspension is not often an issue in bioreactors. By the time complete gas dispersion is achieved with the radial impeller the cells should be fully suspended. Still, it should be mentioned that radial impellers are the least economical for particle suspension at any Re range. Furthermore, since the presence of gas reduces the energy transmitted into the mixture, suspended particles have been observed to settle during gassing [16].

In mammalian cell culture, a primary concern is the susceptibility of the cells to damage due to fluid dynamic generated stresses, which may arise from agitation and aeration, a phenomenon commonly called 'shear damage'. A study on the suspension of microcarriers using Chemineer 3-blade HE-3 impellers which are axially down pumping hydrofoils succeeded in producing homogeneous suspension of the particles at 0.5 x 10^{-3} Wkg^{-1}, which is half the threshold value of specific energy that causes cell damage [17]. The study implied that available impellers can be used in tissue cell culture without necessarily designing new devices with special features for the fragile system.

7 THREE PHASE MIXING

Studies have generally focused on the effect of gassing on solid suspension at low concentration. At low gas flowrates the presence of gas has been observed to aid particle suspension with mixed flow impellers pumping up or down. But at higher flowrates and with the 6DT the presence of gas reduces the impeller pumping capacity. This has the effect of making particles which are already suspended settle again, and a higher speed would be required to produce suspension [16].

8 RHEOLOGICAL EFFECTS

In addition to changes in viscosity, deviation from the Newtonian flow behaviour can dramatically affect the mixing dynamics [18]. Pseudoplastic fluids have viscosities that decrease with increased shearing. In the stirred vessel, such fluids tend to be better mixed in the vicinity of the impeller, but more slowly further away from the impeller due to less shearing action. Fluids with yield stress which require a minimal stress to flow, become stagnant near the stationary vessel wall while the central region is intensely mixed, creating what is known as the "cavern", an effect that can be overcome with the use of larger D/T. Viscoelastic fluids are known to exert normal forces to the shearing force, which in the stirred vessel cause the rotating fluid to be pushed to the centre. The rod climbing "Weissenberg effect" is a result of normal forces, although it diminishes at large scale.

In gassed viscous systems, large cavities tend to form even at low gas flowrates causing more abrupt drop in power. The distribution of the bubbles is bimodal, where very tiny bubbles having high residence time even when they are depleted of oxygen, coexist with large bubbles that quickly leave the system before useful mass transfer takes place [2].

9 SCALING UP

Scaling up is often an issue primarily because parameters do not all change proportionally with respect to each other. A common basis for scaling up the stirred vessel is to maintain geometric similarity and equal specific power input, P_G/V_L (W/m^3). In such a case, for a 5-fold increase in impeller diameter, D, the liquid volume, V_L and therefore P_G, will increase 125 times since V_L D^3 for geometrically similar cases. The speed is then estimated using the Po_G equation, giving $N_{Large\ scale} = N_{Small\ scale} (D_{Small\ scale}/D_{Large\ scale})^{2/3}$, i.e. agitation speed must reduce with increase in scale. Hydrodynamics similarity is not necessarily maintained because the dependence of other parameters on N and D is not the same as for specific power input. If speed is kept constant whilst maintaining geometric similarity, P_G/V_L value will be prohibitively high. Constant Re or impeller tip speed gives unrealistically low P_G/V_L values.

10 CONCLUSION

Mixing in the stirred vessel happens not randomly but through complex interactions of geometric, rheological and energetic factors. For a given scale and mixture properties the choice of impeller determines the success of a process in terms of time and energy requirement and product output. The bioreactor has peculiar needs related to the sensitivity of biological systems to temperature, oxygen supply, nutrient availability, and asepticity. This leads to multiple mixing objectives which can be difficult to satisfy all at the same time. This paper highlights the basics of mixing in the stirred vessel. There has been extensive development in the field over the last decades, and literature published on the subject give details on the various aspects of mixing. ■

REFERENCES

[1] Stanbury, P.F., A. Whitaker and S.J. Hall, "Principles of Fermentation Technology", 2nd Edition, Butterworth Heinemann, 1995

[2] Harnby, N., M.F. Edwards, and A.W. Nienow, "Mixing in the Process Industries", 2nd Edition, Butterworth Heinemann, 1992

[3] Annuar, M.S.M., I.K.P. Tan, S. Ibrahim, K.B. Ramachandran, "Production of Medium-Chain-Length Poly(2-hydroxylalkanoates) from Crude Fatty Acids Mixture by Pseudomonas putida", Trans IChemE, Part C, 85 (C2), June 2007, pp 104-119

[4] Nienow, A.W. and S. Chatwin, Lab Sci. and Tech., 19, 1985

[5] Reserdiz, R., A. Martinez, G. Ascanio and E. Galindo, "A New Pneumatic Bearing Dynamometer for Power input Measurement in Stirred Tanks". Chem. Eng. Technol., 14, 1991, pp 105-108

[6] Ibrahim, S. and A.W. Nienow, "Power Curves and Flow Patterns for a Range of Impellers in Newtonian Fluids: 40<Re<5x105", Trans IChemE, Part A, 73, July 1995, pp 485 – 491

[7] van't Riet, K. and J.M. Smith, "The behaviour of gas-liquid mixtures near Rushton turbine blades", Chem. Eng. Science, 1973, 28, pp 1031-1037

[8] Bruijn, W., K. van't Riet and J.M. Smith, "Power Consumption with Aerated Rushton Turbines", Trans. IChemE., 52, 1974, pp 88-104

[9] Bailey, J.E. and D.F. Ollis, "Biochemical Engineering Fundamentals", 2nd Edition, McGraw Hill International Edition, 1986

[10] Nienow, A.W., "Gas-Liquid Mixing Studies: A comparison of Rushton Turbines with some modern impellers", Trans IChemE, 74, Part A, 1996, pp 417-423

[11] Abdul Aziz, A.R., PhD Thesis, "Hydrodynamics and Mass Transfer Characteristics of Curved Blade Impellers for Multiphase Mixing in Stirred Vessels, University of Malaya. 2007

[12] Tatterson, G.B., "Fluid Mixing and Gas Dispersion in Agitated Tanks", McGraw-Hill, Inc., 548, 1991

[13] Ibrahim, S. and A.W. Nienow, "Particle suspension in the turbulent regime: The effect of impeller type and impeller/vessel configuration", Trans IChemE, Part A, Sept 1996, pp 679 – 688

[14] Ibrahim, S. and Nienow, A.W., "Comparing impeller performance for solid-suspension in the transitional flow regime with Newtonian fluids", Trans IChemE, Part A, 77, Nov 1999, pp 721-727

[15] Zwietering, T.N., Suspending of Solid Particles in Liquid by Agitators, Chem. Eng.Sci., 8, 244, 1958

[16] Ibrahim, S., PhD Thesis, "Comparative Studies of Impeller Performance for Solid-Gas-Liquid Mixing", University of Birmingham, 1992

[17] Ibrahim, S. and A.W. Nienow, "Suspension of Microcarriers for Cell Culture with Axial Flow Impellers", Trans IChemE, Part A, Sept 2004, 82 (A9), pp 1082-1088

[18] Nienow, A.W. and T.P. Elson, "Aspects of Mixing in Rheologically Complex Fluids", Chem. Eng Res Des, 66, 1988, pp 5-15

PROFILE

PROFESSOR SHALIZA BINTI IBRAHIM

Professor Shaliza Ibrahim obtained her PhD in 1992 on Multiphase Mixing in Stirred Vessels from the University of Birmingham, under the supervision of Professor Alvin Nienow, a world renowned expert on mixing. She won the 1991 UK Mixing Research Students Competition, followed by the Inaugural High Commissioner's Award for Academic Excellence in London later that year. Although she has moved on to other research areas, mixing remains close to her heart and she continues conducting study on the subject, both in the fundamental and applied aspects.