UNIT- 3

Mixing and Mass Transfer

Transport phenomena in biological systems is actually influenced by the kinetic behaviour of cells. This can happen on different scales. A connection between the length scale and transport phenomena have to be addressed in biological systems so that the kinetics measurements and models can be developed under conditions which will resemble in some senses encountered in the large scale bioreactors.

Normally, fluid circulations is apparent in a large scale bioreactor. Turbulence levels within the reactor gives an impact to the dissolved oxygen concentration and thus influencing the overall cellular kinetics during the growth. A method which is commonly applied to tackle such a problem is mixing. It is a physical operation that reduces non- uniformities in fluid by eliminating gradients of concentration, temperature etc. This is done by interchanging material between different locations to produce a mingling of components. For a perfectly mixed system, there is a random homogeneous distribution of system properties that involves:

- dispersing gas (O_2, CO_2, air etc) through liquid in the form of small bubbles (smaller bubbles leads to higher mass transfer rate).
- maintaining suspension of solid particles, for instance immobilised enzymes/cells or the cell itself.
- blending soluble components of the medium such as sugars.
- dispersing immiscible liquids to form an emulsion/suspension of fine drops
- promoting/maintaining consistent heat transfer to or from the liquid.

Mixing can be divided into 2 parts:
1. macro-mixing
2. micro-mixing

Macro-mixing

The process of mixing on the scale of the whole vessel or reactor is termed as macromixing. Macro-mixing is characterized by the residence time distribution, (RTD) for a continuous flow system. It is actually the time spent within the boundaries of the system i.e. the time between the inlet and outlet for a certain volume element (exceptional for ideal plug flow reactor where all volume elements leave the system with different RTD).

Micro-mixing

Micromixing consists of the viscous –convective deformation of fluid elements followed by molecular diffusion. Micromixing is a process in which ingredient particles rearrange to form a blend. Development of pharmaceutical formulations requires understanding how the ingredients blend with each other and how the blending progresses through different stages.

Methods for Characterising Mixing

The simplest method of characterizing mixing is by tracer techniques in which a tracer is added to the bioreactor and its concentration is measured as a function of time. The techniques include,

1. conductivity method based on electrolytes as tracer:
   - inexpensive
   - easily implemented
   - disadvantage: (most media used for fermentation are good conductors { poor sensitivity)

2. pH method based on acids or bases as tracer
   - easily implemented
   - measuring change of pH after addition of base(acid), therefore mixing time can be determined.
   - method can be applied under real process conditions
   - microbial activity may influence the results since many microorganisms produce acids as metabolic products (it is important that the mixing time is smaller than the characteristic time for acid production)
   - disadvantage: most fermentation media have a high buffer capacity and large pulses are required in order to obtain good sensitivity.

3. fluorescence method based on fluorophores as tracer
   - based on measurement of an inert fluorophore such as NADPH, riboflavin or coumarin.
   - possible to quantify mixing time under real process conditions
   - for many fermentation media, the background fluorescence is high and the sensitivity may therefore be poor

4. isotope method based on radioactive isotopes as tracer.
   - based on addition of radioactive isotopes and measurement of radioactivity using scintillation counters
   - advantage: sensor can be placed outside bioreactor
   - disadvantage: radioactive effect caused to the product formed

Gas-Liquid Mass Transfer

For the gas-liquid mass transfer, the interface between the gas and liquid phases is shown in Figure . This is normally modelled by the two-film theory first introduced by Whitman
Phase boundary

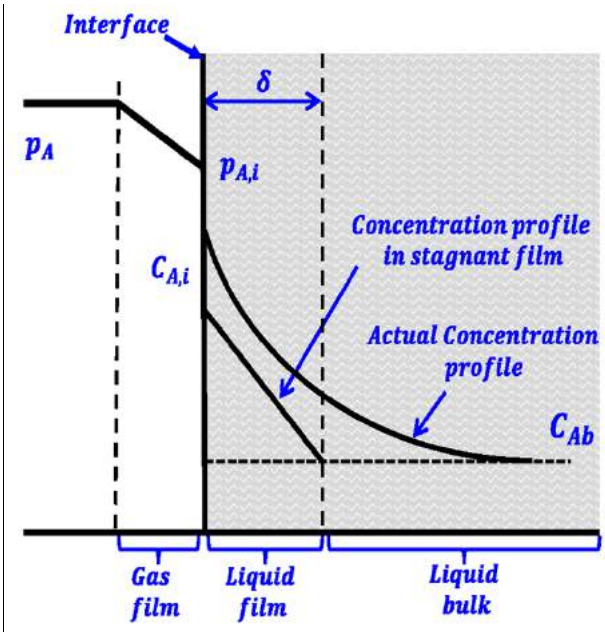


Figure : Concentration gradient for gas-liquid mass transfer.

in 1923. The flux JA of compound A through each of the two films is described as theproduct of the concentration difference across the film layer, which is the linear driving force and the mass transfer coefficient. This also defines the Fick's Law of diffusion, where cA is the concentration of compound A.

In a dilute aqueous solutions, the concentration of the each sides of the gas-liquid interface is related by Henry's Law;
pAi = HAcAi
where HA is the Henry's constant with units of atm.L.mol-1.

Oxygen Transfer Rate

Mass transfer in biological processes is important especially for the gas-liquid system.A sparingly soluble gas such as oxygen is transferred from a rising air bubble into a liquid phase containing cells. The gas molecules must pass through a series of transport resistances, the relative magnitudes of which depend on;

- bubble hydrodynamics,
- temperature,
- cellular activity and density,
- solution composition,
- interfacial phenomena and other factors.

Oxygen Consumption in Cell Growth

In most aerobic cultures, cells obtained the oxygen supply from the liquid. The rate of transfer of oxygen from gas to liquid is highly important especially when there is a high cell density in a particular fermentation {this leads to the oxygen limited growth}. The rate of oxygen transfer per unit volume of fluid is given by NA. The solubility of oxygen in aqueous solutions at ambient temperature and pressure is about 10 ppm and such a value can be easily consumed in aerobic cultures. Therefore, continuous supply of the gas is important. Design of fermenters for aerobic operation must take these factors into account and provide optimum mass transfer conditions.

Factors Affecting Cellular Oxygen Demand

The rate of oxygen consumed by cells in a fermenter determines the rate at which the oxygen must be transferred from gas to liquid. Factors that influence the oxygen demand include:

1. type of bacterial used (species)
2. culture growth phase
3. nature of carbon source

Measurement of kla in Continuous-Stirred-Tank Bioreactor and Airlift Bioreactor

Continuous-Stirred-Tank Bioreactor

Mass transfer coefficients for oxygen are normally determined experimentally. The methods include:

1. Oxygen-Balance Method
2. Dynamic Method
3. Sulphite Oxidation

Oxygen-balance method8.5) for gas-liquid mass transfer. For oxygen transfer;

NO2 = kla(c *O2- cO2)

The steps of carrying the experiment is given as follows:
1. Measuring the content of the inlet and outlet flow of oxygen
2. The difference in oxygen flow between inlet and outlet should be equal to the rate of oxygen transfer from gas to liquid at steady-state. The difference between the two flowrates above results in the rate at which oxygen is transferred out of the gas into the liquid represented by NO2 .
Since the concentration of gases is normally measured by their partial pressure given by;
pV = nRT
n/V =p/RT
since c = n/V
3. Measure the partial pressure of oxygen at the inlet and outlet streams as well as their temperatures in order to determine NO2 . This value is then used together with O2 and cO2 to calculate kla. Methods of determining c *O2 and cO2 are given below:

(a) method of determining saturation constant of dissolved oxygen or oxygen solubility refers to the driving force for the oxygen transfer. The value of driving force is usually small and thus, the saturation constant have to be found accurately.

- Monomeric-volumetric method
- Mass spectrometric method
- Gas chromatographic method
- Chemical method
- High pressure/high temperature method

(b) method of determining dissolved-oxygen concentration (cO2): The dissolved-oxygen concentration in media is normally measured using a dissolved-oxygen electrode. Two types of electrodes are available;

i. galvanic electrode
ii. polarographic electrode

- for both types, a membrane which is permeable to oxygen separates the fermentation fluid from the electrode. Oxygen diffuses through this membrane to the cathode where it reacts to produce a current between anode and cathode proportional to the oxygen partial pressure in the broth/media.
- the electrolyte solution in the electrode supplies ions which take part in the reactions and must be replenished at regular intervals.
- supply of oxygen molecules from the bulk medium in the vessel to the cathode within the probe is in itself a mass-transfer process.
- this happens because there is no liquid motion in the membrane or electrolyte solution and little motion in the liquid film at the membrane interface{operation of the probe relies on diffusion of oxygen across these thicknesses.}
- due to such diffusion processes, the response of the probe (electrode) to sudden changes in dissolved-oxygen level is subject to delay.
- electrode can be improved if the bulk liquid is stirred rapidly{therefore decreases the thickness of the liquid film at the membrane surface.}
- the smaller the size of cathode and low rate of oxygen consumed by the probe{means that their response is quicker}so that it can be used to measure dissolved-oxygen levels in un-agitated systems.
- repeat calibration of the probe if necessary before use.
- precaution: do not leave probe in the fermentation broth for longer period{this can cause fouling due to cells attaching to the membrane surface}.

Dynamic method

Suitable with the name, this technique of obtaining the mass transfer coefficient is based on the transient state of oxygen balance. The cost of equipment required to undertake this method is low (one of the advantages). Consider a fermenter containing cells in a batch configuration;

- at time t = 0, the broth is de-oxygenated (by sparging nitrogen gas into the fermenter or by stopping the air °ow if the culture is oxygen- consuming). The drop of the dissolved oxygen concentration cO2 can be determined.
- when the level is about half of the critical value (precaution should be taken so that the level does not drop below this critical velue), air is pumped back into the vessel at a constant flowrate. The increase in the dissolved-oxygen concentration is monitored.
- by assuming that the re-oxygenation of the media is fast relative to the growth, the dissolved-oxygen concentration will reach a steady-state value, cO2 . This shows the balance between the oxygen supply and oxygen consumption in the system.

- cO2,1 and cO2,2 are two oxygen concentrations measured during the re-oxygenation at times t1 and t2 respectively. From these information, a transient equation for the rate of oxygen dissolved in the media can be developed.

$$dcO2/dt = kla(c^*O2 - cO2) - qO2x$$

where, qO2x is the rate of oxygen consumed by the cells which can be determined by considering the final steady dissolved-oxygen concentration cO2 . for this technique, an oxygen probe with fast response time is needed to measure cO2 otherwise, accurate result cannot be obtained.

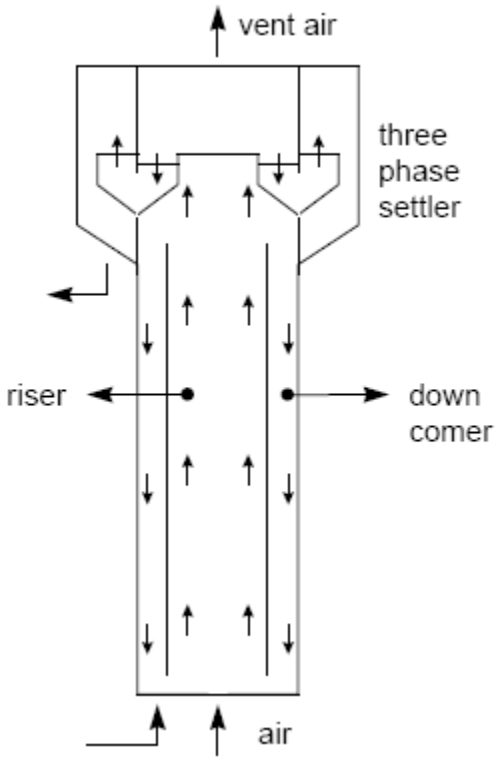
- the average residence time of the gas in the system can also affect the accuracy of this method. This occurs during the switching of de-oxygenation to the aeration at the start of the measurement. There is a nitrogen hold-up within the vessel when air is re-introduced{measuring cO2 , does not reflect the kinetics of simple oxygen transfer until a hold-up of air is established (longer time is needed for large vessel}. For convenience, this method is only suitable for vessels with height less than 1 m.
- the technique also is not suitable for viscous media/broths for a similar reason; longer residence times of bubbles in viscous media affect the accuracy of the measurement.

Sulphite oxidation method

This method is based on the oxidation of sodium sulphite to sulphate in the presence of a catalyst such as copper ions, Cu+. Such a method has been found to give higher values of kla compared to other techniques.

Airlift Bioreactor

The configuration of the airlift bioreactor is shown in Figure .



The method of determining the mass transfer coefficient can be done in 2 ways;
1. Dynamic method
2. Sulphite oxidation method

Sulphite method

The method is based on the modified sulphite method. Throughout the work, the temperature was controlled at 37±C.The oxygen probe was installed in the riser section during the runs and the air flowrate was set in a range of different values from 0.1 to 1.5 vvm, fixed for each run. It is impossible to distinguish differences in the oxygen transfer coefficient in the riser and the downcomer sections using the sulphite method.

Dynamic method

The determination of kla using the dynamic method was described by Ruchti et al.(1981).The volumetric oxygen transfer coefficient was measured in two different position, in the riser, and in the downcomer and the air flowrate was varied between 0.1 and 1.5 vvm. The study was divided into 2 parts, the mass transfer coefficient for the riser section and that for the downcomer section. Since air was introduced into the bioreactor directly in the riser, this section is supposed to have a better mass transfer, leading to kla values higher than in the downcomer for a given temperature. It was observed that there were more bubbles leaving the liquid in the upper section of the reactor than going down in the downcomer section. Bello et al. (1985) have reported that the oxygen concentration inside the bubbles in the downcomer is lower than the one in the riser.

Liquid Mixing

The main reason of applying mixing in fermentation processes is to reduce the uniformities in fluid by elimination of the gradients of concentration, temperature and other properties. Such process can be accomplished by interchanging material between different locations to produce a mingling of components. For a perfectly mixed system, there is a random homogeneous distribution of system properties which involves:

- blending soluble components of the medium
- dispersing gases, for instance, air through the liquid in the form of small bubbles
- maintaining suspension of solid particles (cells or immobilised enzymes)
- dispersing immiscible liquids to form emulsion/ fine drops
- promoting heat transfer to or from liquid

If mixing does not maintain a uniform suspension of biomass, substrate concentrations can quickly drop to zero in areas where cells settle out of suspension. Bioreactors must be capable of transferring heat to or from the broth quickly enough such that the desired temperature is maintained. The usual way in achieving mixing of components is by mechanical agitation using impeller.

Types of Mixing and Stirrers

Mixing:

Mixing is usually carried out in a stirred tank reactor which has been fitted with baffles. The normal shape of a reactor is cylindrical that can avoid unreachable region such as sharp corners and pockets. This also discourages formation of stagnant regions. Mixing is achieved using an impeller mounted in the tank for use with Newtonian fluids{ratio of tank diameter : impeller diameter is about 3:1.}

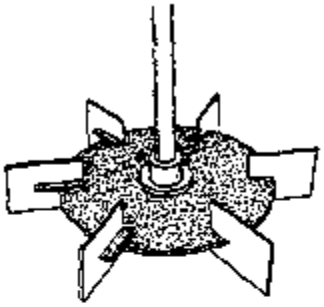
Impeller:

Impeller is normally positioned overhead on a centrally located stirrer shaft and driven rapidly by the stirrer motor. Liquid is forced away from the impeller, circulates through the vessel and periodically returns to the impeller. Depth of liquid in the tank should be no more than 1.0-1.25 times the tank diameter.Some impellers have flat blades, propeller type and helical screw.

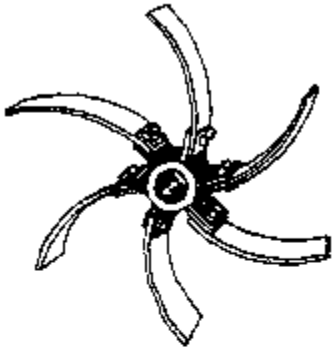
Choice of impeller depends on;
1. viscosity of liquid
2. sensitivity of systems to mechanical shear.

- for low to medium viscosity liquids{propeller and flat-blade turbines are recommended.
- frequently used impeller for industrial fermentation{6-flat-blade disc-mounted turbine (Rushton turbine) is used.

Radial Flow Impellers

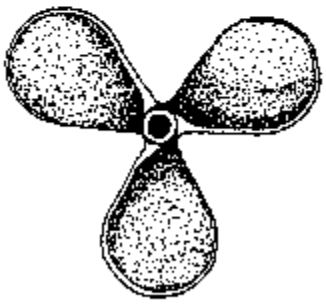


Disk Style Flat Blade Turbine
Commonly Referred to as
the Rushton Impeller

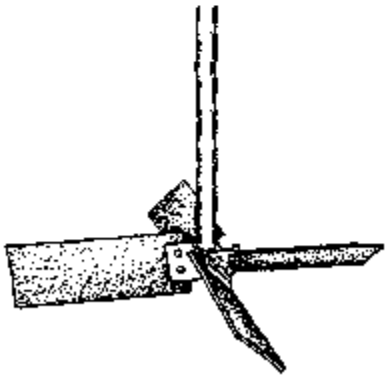


Sweptback or Curved Blade Turbine
(a Spiral Turbine)

Axial Flow Impellers



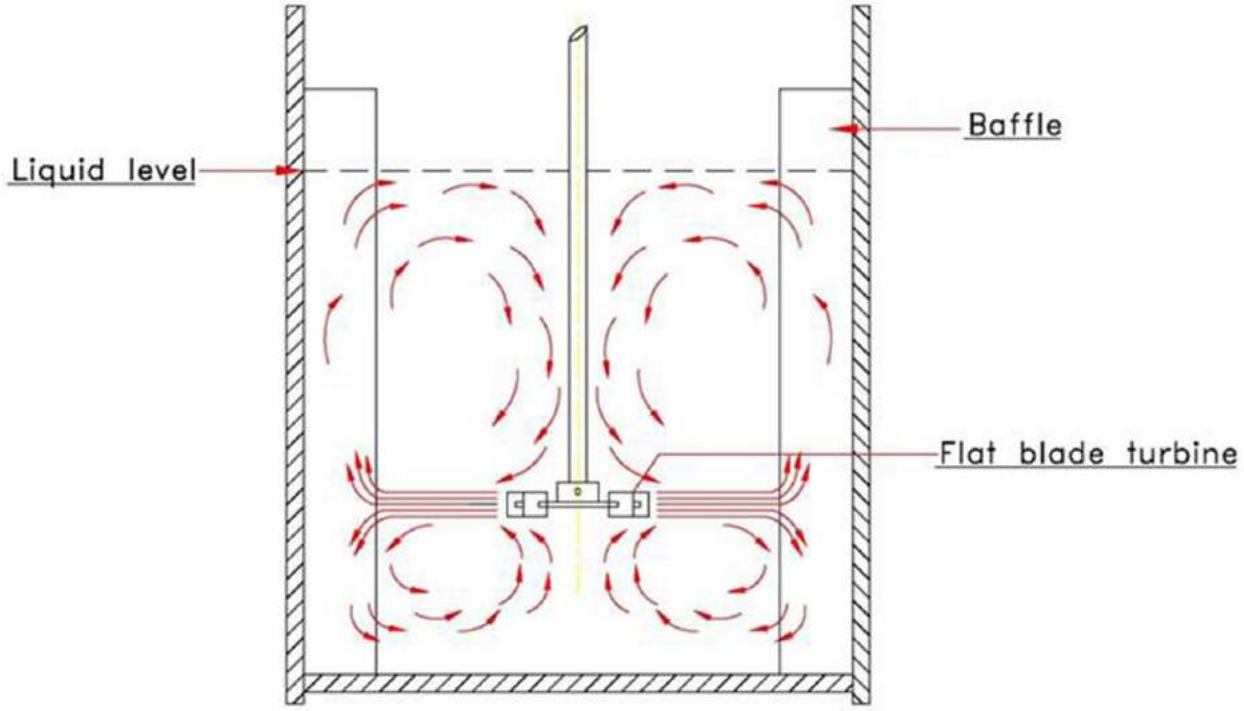
Propeller



45° Pitched Blade Turbine

Baffles:

Strips of metal mounted against the wall of the tank installed to reduce vortexing and swirling of liquid. It is attached to the tank by means of welded brackets{4 equally-spaced baffles are usually sufficient to prevent vortex formation.} Optimum baffle width depends on the impeller design and fluid viscosity {of order D/10 to D/12 of the tank diameter. }It can be mounted away from wall with clearance of about D/50 of the tank diameter. This will prevent sedimentation and development of stagnant zones at the inner edge of the baffle during mixing of viscous cell suspensions.



Types of Flow in Agitated Tanks

Flow pattern in a stirred-tank depend on:
1. the impeller type/design
2. properties of fluid
3. size and geometric proportions of vessel, baffles and impeller

The flow can be divided into 3 different patterns:
1. circular flow
2. radial flow
3. axial flow

For circular flow:

- rotational in action
- simple flow
- movement of liquid in streamline fashion{gives little mixing between fluid at different heights in the tank}
- can lead to vortex development
- at high speed{vortex may reach the impeller so that gas from the surrounding atmosphere is drawn to the liquid
- generally disadvantageous and should be avoided
- can be prevented by installing baffles that interrupt such a flow and create turbulence

For radial flow:

- this type of flow is produced from impellers that have blades which are parallel to the vertical axis of the stirrer shaft and tank

example: 6-flat-blade disc turbine impeller

- liquid is driven radially from the impeller against the walls of the tank{divided into 2-stream, one flowing upwards to the top of the tank and the other flowing downwards toward the base of the tank
- both streams reach the central axis of the tank and are drawn back to the impeller
- this type of flow also produces circular flow and it has to be avoided with baffles

For axial flow:

- this type of flow generally produced by impeller with blades that have an angle of less than 90 º to the plane of rotation and promote axial top to bottom motion
- propellers are type of impeller that give axial flow in a stirred-tank fluid leaving the impeller is driven downwards until it is deflected from the base of the tank
- it then spreads out over the floor and flows up along the wall before being drawn back to the impeller
- such impellers are particularly useful when strong vertical currents are required especially when dealing with mixing of solid particles{strong axial flow leaving the impeller will avoid the solid from settling at the bottom of the tank}

The Mechanism of Mixing

An effective mixing of fluid can be achieved when;

- sufficient velocity of fluid which can carry material into most parts of the tank
- turbulent flow for perfect mixing

These factors are a combination of physical processes that include;

- distribution (process which comprises of macromixing) during the mixing of dye, the process of transporting the dye to all regions of vessel by bulk distribution currents is termed distribution
- the process can be relatively show, for large vessel, the size of the circulation paths is also large therefore leads to long period of time for the flow to traverse{this inhibits rapid mixing}
- distribution is the slowest step in mixing process, for high rotational speed impeller ,the process will be turbulence (this only happens when fluid travels randomly or erratically in the form of cross-currents)
- kinetic energy from the turbulence fluid is directed into regions of rotational flow known as eddies
- dispersion (either macro or micro mixing), process of breaking up bulk flow into smaller eddies.It facilitates rapid transfer of material throughout the vessel, the size of eddies that limits the degree of dispersion is give approximately by the Kolmogorov scale of mixing or also known as scale of turbulence.
-

$$\gamma = (\nu^3 / \varepsilon)^{1/4}$$

where γ is the characteristic dimension of the smaller eddies, ν represents the kinematic viscosity of the fluid and ε is the local rate of turbulence energy dissipation per unit mass of liquid

- diffusion process which comprises of micromixing, molecular diffusion is generally regarded as slow process{ over small distances, the process can accomplish quite rapidly,

for eddies of diameter 30 to 100 μm, homogeneity is achieved in about 1 s for low viscosity fluids

- if power input to a stirred-tank can produce eddies of the given dimensions, mixing on a molecular scale is then accomplished

Power Requirement for Mixing

In order to drive the impeller for mixing, electrical power is usually used. For a given stirrer speed, the power required depends on the resistance offered by the fluid to rotation of the impeller. The average power consumption per unit volume for industrial bioreactors;

- 10 kWm^{-3} (small vessel)
- 1-2 kWm^{-3} (large vessel)

Friction from the motor (gearbox and seals) also contribute to loss of energy transmitted to the fluid, thus, the electrical power consumed by the motor is greater than the mixing power which depends on the efficiency of the drive.

Methods of calculating power requirements during mixing can be divided into 2 types:
1. un-gased Newtonian fluids
2. un-gased non-Newtonian fluids
3. gased fluids

Un-gased Newtonian Fluids

For non aerated fluids, the mixing power depends on,

- stirrer speed
- impeller diameter and geometry
- fluid properties (density, viscosity etc.)

These can be expressed in terms of the dimensionless numbers, for instance, Reynolds number, Re and Power number, Np;

$$Re = \frac{\rho V D}{\mu}$$

$$N_p = \frac{P}{\rho N^3 D_a^5}$$

where P is the power, ρ is the fluid density, Ni refers to the stirrer speed, di represents the diameter of the impeller (while D represents pipe diameter), v is the fluid velocity and gives the viscosity of the fluid.

These two correlations has been determined experimentally for different range of impellers and agitated tank configurations. Then the power number of a particular impeller is found, the power required can be determined by rearranging equation which gives;

$$P = N_P \rho N^3 Di^5$$

The two correlations also depend on the flow regimes within the tank (either in the region of laminar flow, transition flow or turbulent flow):

- for laminar regime, ($Re < 10^1$) is for a number of impellers, ($Re > 10^2$) is for stirrers with very small wall-clearence such as the anchor and helical-ribbon
- in this regime, the power number is inversely proportional to the Reynolds number;
- power required for laminar flow is independent of the density of the fluid however it is directly proportional to fluid viscosity
- for transition regime between laminar and turbulent flow, both density and viscosity affect power requirements
- there is usually a gradual transition from laminar to fully-developed turbulent flow in

agitated tanks
The power required for turbulent flow is independent of the viscosity of the fluid, but proportional to the fluid density.For most small impellers in baffled tanks, turbulent regime is fully developed at Re > 10^3 or 10^4.For tanks without baffles, turbulent is not fully developed until Re > 10^5

Un-gased non-Newtonian Fluids
For non-Newtonian fluids, the power estimation is more difficult. Viscosity of such fluids varies with shear conditions, the impeller Re used to correlate power requirements must be re-defined.
A few correlations has been developed based on the apparent viscosity.

Gased Fluids

Apparently, when gas is sparged in a liquid, the hydrodynamic of the liquid around the impeller (or any fluids) immediately changes. This reduces the power requirements of the mixing when the density of the fluid decreases. Large gas-filled cavities developed behind the stirrer blades in gased liquids will reduce the resistance to the fluid flow and decrease the drag coefficient of the impeller.The average deviation between the two systems is approximately 12%{with sparging}, the power consumption could be reduced to as little as 50% of the un-gased value which also depends on the flowrate of the gas.